# Interleukin-1β and interleukin-6 affect electrophysiological properties of thalamic relay cells

Vinicius Nikolaos Samios and Takafumi Inoue

Department of Life Science and Medical Bioscience, Waseda University, Tokyo, Japan

E-mail: samios@moegi.waseda.jp and inoue.t@waseda.jp

Correspondence to: Dr. Takafumi Inoue
Department of Life Science and Medical Bioscience
Waseda University,
2-2 Wakamatsu-cho, Shinjuku,
Tokyo-162-8480, Japan
tel: +81-3-5369-7328
e-mail: inoue.t@waseda.jp

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**Abstract**

By acknowledging the relation between brain and body in health and disease, inflammatory processes may play a key role in this reciprocal relation. Pro-inflammatory cytokines, such as interleukin-1β (IL-1β) and interleukin-6 (IL-6) are some of the agents involved in those processes. What exactly is their role in the CNS however is not that clear so far. To address the question of how pro-inflammatory cytokines may affect information processing at the cellular and molecular levels, relay neurons in the thalamic dorsal lateral geniculate nucleus in mouse brain slices were exposed to those cytokines and studied with the patch-clamp technique. IL-1β promoted hyperpolarization of the resting membrane potential ($V_{rest}$), decrease of input resistance ($R_{in}$), decrease of $I_h$ rectification, decrease in action potential (AP) threshold and decrease in the number of APs in low threshold calcium spike (LTS) bursts, while IL-6 promoted decrease of $R_{in}$ and decrease in the number of APs in LTS bursts. Computer simulations provided candidates for ionic conductance affected by those cytokines. Collectively, these findings demonstrate that IL-1β and IL-6 have modulatory effects on electrophysiological properties of thalamic neurons, implying that the thalamic

functions may be affected by systemic disorders that present with high levels of those cytokines.

**Keywords**: thalamic relay cell; interleukin; IL-1β; IL-6; patch-clamp

**Abbreviations:**

| | |
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| AP: | action potential |
| dLGN: | dorsal lateral geniculate nucleus |
| ISI: | inter-spike interval |
| LTS: | low threshold calcium spike |

## Introduction

Clinical settings where a systemic condition is directly related to abnormal behavior are very common. Metastatic cancer, for instance, is often manifested with depression in adults (Miller et al., 2008), as well as major surgery and sepsis commonly relate with delirium and depressed sensorium (Gofton and Young, 2012; O'Regan et al., 2013). On the other hand, patients with neuropsychiatric conditions such as mood disorders (Crump et al., 2013), schizophrenia (Fan et al., 2013) and autism (Tyler et al., 2011) have an increased chance of developing a systemic condition when compared to their healthy pairs. By acknowledging the relation between brain and body in health and disease, a natural question concerning the mechanisms by which that phenomenon takes place is generated. Among different possibilities, the inflammatory hypothesis, i.e., inflammation and its subproducts might have a direct effect in the etiology of those pathologic conditions, has attracted attention (Berk et al., 2013).

When inflammatory processes take place in the brain, pro-inflammatory cytokines, such as interleukin-1β (IL-1β) and interleukin-6 (IL-6), among others seem to play major roles (Teeling and Perry, 2009). Recent evidence has also been

demonstrating that systemic and central levels of pro-inflammatory cytokines are increased in neurological and psychiatric conditions (Careaga et al., 2010; Friedman and Dingledine, 2011; Miller et al., 2009). What exactly is the role of those cytokines in the central nervous system (CNS) however is not that clear so far.

IL-1β belongs to the IL-1 family of cytokines, and both IL-1β and its mRNA are found at low levels in specific areas of the CNS, being prone to induction when several types of stress occur (Gądek-Michalska and Bugajski, 2010; Gosselin and Rivest, 2007). Hypothalamic and hippocampal neurons were shown to be affected by IL-1β through the prostaglandin E2 signaling upon activation of neuronal IL-1 receptors (Ferri and Ferguson, 2003; Roozendaal et al., 2009).

IL-6 is a four-helix bundle cytokine, founder of a group of cytokines called neuropoietins, which are structurally related and share a common signal transducer, gp130. IL-6 is known to exert its effects through three different pathways, namely JAK-STAT, MAPK and PI-3K/Akt pathways, even though the last one has not been well described in the CNS (Erta et al., 2012; Spooren et al., 2011). IL-6 and its receptor, IL-6R, are expressed in neurons and glial cells at different levels in several brain areas

(Gadient and Otten, 1994, 1993; Schöbitz et al., 1993), and their actions may be as varied as inducing the cholinergic phenotype in sympathetic neurons (Fann and Patterson, 1994; März et al., 1998; Oh and O'Malley, 1994) to affecting adult neurogenesis (Bauer et al., 2007; Deverman and Patterson, 2009).

To address the question of how IL-1β and IL-6 affect information processing at the cellular and molecular levels, the thalamus, which is a brain area comprised of several nuclei related to sensory information processing and relaying, as well as to regulation of the consciousness level, was chosen as the target brain area of this study.

Thalamic neurons are usually divided into two major categories: relay neurons, which directly receive inputs from sensory afferents and send projections to the cortex, the so called thalamo-cortical projections, and interneurons, which only handle local connections, believed to exert modulatory functions inside the thalamus (McCormick, 1992; Williams et al., 1996). Thalamic relay neurons show diverse electrophysiological properties. At membrane potentials more positive than -55 mV, depolarization promotes a tonic firing of action potentials. However, at membrane potentials more negative than -60 mV depolarization induces so called low threshold

calcium spikes (LTS) through activation of the T-type $Ca^{2+}$ channels (Cav3.1–3.3), which are usually crowned with a burst of action potentials (Chemin et al., 2002).

In spite of the importance of thalamic circuitry in sensory information processing, as well as in the regulation of states of vigilance, little work has been conducted to address if and how cytokines may affect its functions. Here, by using the patch-clamp technique combined with computational simulation, modulatory actions of cytokines IL-1β and IL-6 were assessed and described in thalamic relay neurons from mice for the first time.

**Materials and methods**

Animal care was performed in accordance with guidelines outlined in the Institutional Animal Care and Use Committee of Waseda University. The protocol was approved by the Committee on the Ethics of Animal Experiments of Waseda University. Throughout the experimental procedures, all efforts were made to minimize the number of animals used and their suffering. Mice were fed *ad libitum* with standard laboratory chow and water in standard animal cages under a 12 h light/dark cycle.

Experiments were performed in brain slices prepared from C57BL/6J mice. Animals aged 8 ± 2 weeks, both male and female, were quickly decapitated and the whole brain was removed and placed in an ice cold choline based solution (in mM: CholinCl 120, KCl 3, $NaH_2PO_4$ 1.25, $NaHCO_3$ 26, $MgCl_2$ 8, Glucose 20) for 2 minutes, a block containing the thalamus was cut from the brain, and brain slices of 300 μm thickness containing the dorsal lateral geniculate nucleus of the thalamus (dLGN) were prepared using a vibratome-type slicer (Linearslicer Pro 7, Dosaka EM, Kyoto, Japan). The slices were then kept in a chamber containing artificial cerebrospinal fluid (ACSF)

bubbled with a 95% $O_2$ and 5% $CO_2$ gas mixture and let to rest in room temperature (about 24°C) for at least 1 hour before experiments. The ACSF consisted of (in mM): NaCl 125, KCl 2.5, $NaH_2PO_4$ 1.25, $NaHCO_3$ 26, $MgCl_2$ 1, $CaCl_2$ 2 and glucose 20, with an adjusted osmolality of 310-315 mOsm.

During experiments, slices were kept submerged in a chamber mounted over the stage of an upright microscope (BX50WI, Olympus, Tokyo, Japan). The chamber was continuously superfused with ACSF at the rate of 4.5 ml/min at 32°C.

Whole cell current-clamp recordings were made from relay neurons in dLGN. The cells were visualized with differential interference contrast optics using infrared light. Relay neurons were distinguished from local interneurons by their electrophysiological characteristics (lower input resistance and robust low threshold calcium spikes (LTS) crowned with a burst of action potentials when hyperpolarized). Only cells with a resting potential more negative than -60.5 mV were used for this study. Borosilicate glass electrodes (2.5-5 MΩ) filled with an internal solution consisting of (mM): potassium gluconate 130, KCl 5, NaCl 5, HEPES 10, $MgCl_2$ 1, $Na_2ATP$ 4,

NaGTP 1 and EGTA 0.4 were used. The pH was adjusted with KOH to 7.25 and the osmolality to 308 mOsm.

The electrical signals were amplified and low-pass filtered at 2 kHz with an Axopatch 1D amplifier (Molecular Devices, Sunnyvale, CA, USA). Membrane capacitance and series resistance were cancelled with built-in circuitry of the amplifier and series resistance was periodically checked. Cells either showing series resistance higher than 20 MΩ or a variation greater than 30% during recording were discarded. Data was digitized at 10 kHz with a PCIe-6259 interface (National Instruments, Austin, TX, USA) with a Mac computer running in-house software, TI Workbench, written by T.I.

Before starting recordings, cells were left to stabilize their resting potential for at least 5 minutes and at most 10 minutes after establishing whole-cell mode patch clamp (break-in). The experiment was carried out in I-Clamp mode at all times and consisted of a square current protocol followed by a ramp current protocol. During the square current protocol, square current pulses were injected for 1.5 seconds with an interval of 3 seconds. The square pulses consisted of fixed current increments, which

led membrane potential ($V_m$) ranging from -100 mV to a potential that was saturated by action potentials. The ramp protocol consisted of a depolarizing current ramp delivered at resting potential ($V_{rest}$) aiming at assessing the action potential threshold. Each neuron was subjected to the protocol pair for four times during the whole experiment, and delivery of each protocol pair was spaced with a 10 minutes interval. During the first protocol pair, cells were only exposed to the normal ACSF, to which we refer as the "baseline" period in this document. Immediately after, the bath superfusion was switched to ACSF containing one of the cytokines or ACSF alone as a control. The brain slice was left exposed to the cytokine bath for 10 minutes before delivery of the second I-Clamp protocol pair. After another 10 minutes of continuous exposure to the cytokine without stimulation, the current injection protocol pair was repeated for the third time, after which the superfusion solution was again switched to the regular ACSF for washing the cytokine. After 10 minutes of washing, the current injection protocol pair was repeated for the fourth and last time.

Cytokines were purchased from PeproTech (Rocky Hill, NJ, USA), Prospec (Ness Ziona, Israel) or Milteny Biotec (Bergisch Gladbach, Germany). These peptides

were dissolved in water at 100 μg/ml and stored in aliquots at -80°C. IL-1β and IL-6 were added to the superfusing ACSF at final concentrations of 1.4, and 1.0 nM, respectively. The choice of cytokine concentrations was based on previous patch-clamp studies on brain slices (Ferri et al., 2005; Kawasaki et al., 2008; Oh et al., 2010), being much higher than the levels found in physiological conditions (<10 pM) (Bernardes et al., 2013; Söderlund et al., 2009) but in accordance with the high levels found in pathologic situations, such as trauma (Taupin et al., 1993), meningitis (van Deuren et al., 1997) and lipopolysaccharide (LPS) administration (Erickson and Banks, 2011).

Offline data analyses were done with TI Workbench, and statistical analyses were performed with SPSS (IBM software) and R (Vienna University of Economics and Business). Results are given as means ± SEM, unless otherwise specified. The statistical difference among groups was determined using an ANOVA test followed by a HSD Tukey post-hoc test when a significant result at the level of 95% was found in the ANOVA test. When samples didn't match a normal distribution, a non-parametric Kruskal-Wallis ANOVA test was performed.

Computer simulations were performed with the NEURON software. For that purpose, a previously described single-compartment thalamo-cortical neuron model (Huguenard and McCormick, 1992; McCormick and Huguenard, 1992) adapted to NEURON (McCormick, 1992; Meuth et al., 2005) was used. The mathematical descriptions of $I_{hh}$, $I_A$, $I_{K2}$, $I_C$, $I_L$, $I_T$, $I_{NaP}$ and $I_h$ were used as the basis for this model of thalamic relay cell, thus making it capable of displaying the two typical firing modes of action potential found in those cells: the tonic mode and the LTS bursting mode. The inward rectifying current $I_{Kir}$ (Williams et al., 1997) was also added to this model in order to better reproduce the recordings of real cells. The general equation describing $V_m$ over time is

$$C_m(dV/dt) = I_{inject} - \Sigma I_{cell}$$

where $C_m$ is the membrane capacitance, $\Sigma I_{cell}$ is the sum of the currents mentioned above and $I_{inject}$ is the injected current. The noninactivating current $I_{Kir}$ was modeled through the following expression (Williams et al., 1997)

$$I_{Kir} = g_{Kir} m^a (V - E_{Kir})$$

where $g_{Kir}$ is the maximal conductance of the current $I_{Kir}$, whereas $m$ is the activation variable, and $a$ is its exponent (=1). $E_{Kir}$ is the equilibrium potential of $I_{Kir}$.

Simulations were performed assuming maximal conductance and fixed equilibrium potentials, which were systematically changed in order better fit empirical data. All computer modeling was carried out with temperature setting at 32°C.

**Results**

Data from a total of 42 cells were used for this study. Basic membrane properties of these cells before administration of ligands were compared among cell groups that would be exposed to each agonist. In the control group, no agonist was added to the superfusing ACSF throughout the experiment. Membrane capacitance was not different among control, IL-1β and IL-6 groups (51.5 ± 2.4, n=22, 50.9 ± 3.6, n=11 and 57.7 ± 4.6 pF, n=9), respectively, $p>0.05$, one way ANOVA). Input resistance ($R_{in}$) was also similar among the three groups (211 ± 12, 198 ± 13 and 231 ± 17 MΩ, respectively, $p>0.05$).

*Resting membrane potential ($V_{rest}$)*

Averages of resting membrane potential ($V_{rest}$) at baseline were -64.6 ± 0.6, -64.4 ± 0.5 and -63.7 ± 0.7 mV for the control, IL-1β and IL-6 groups, respectively. A one way ANOVA test did not show any difference among groups ($p>0.05$). Further comparisons were done with the difference of each cell's $V_{rest}$ from its respective baseline value ($\Delta V_{rest}$, Fig. 1). After 10 minutes of exposure to the cytokines, no

difference was found among the three groups. However, after 25 minutes, the IL-1β group was significantly more hyperpolarized (-1.7 ± 1.3 mV, n=8, Fig. 1B) than the control group (3.2 ± 0.9 mV, n=16, $p<0.05$, HSD Tukey test). Cells exposed to IL-6 (n=7) did not show any significant change in $V_{rest}$ when compared to the other groups. After washing the cytokines, no difference in $V_{rest}$ was detected anymore.

*Input resistance ($R_{in}$)*

For each cell, a current-voltage (I-V) curve was drawn by taking peak potentials resulting from step-wise square current pulse commands (Figs. 2A and B). The input resistance ($R_{in}$) was measured as the slope of the I-V curve taken between the point corresponding to the resting potential and the point one step hyperpolarized to that. Comparison of normalized means of $R_{in}$ showed that after 10 minutes of exposure to the cytokines, a significant difference was found upon comparison of the three groups (one way ANOVA, $p<0.05$, Fig. 2C), and a post-hoc test showed significant decrease of $R_{in}$ in both the IL-1β and IL-6 groups (-16 ± 4%, n=10, $p<0.05$ and -18 ± 6%, n=7, $p<0.05$, respectively) compared to the control group (-4 ± 3%, n=21). After 25 minutes of

exposure, a persistent decrease in $R_{in}$ was still found, and the IL-6 group (-33 ± 6%, n=7) showed a significant dicrease in $R_{in}$ compared to the control group (-5.8 ± 5%, n=16, $p<0.05$). No difference was found between other pairs. After 10 minutes of washing the cytokines, no statistically significant difference was seen anymore among the three groups.

*Inward rectification attributable to $I_h$ current ($I_h$ rectification)*

Every cell was hyperpolarized to -100 mV with a rectangular current pulse, a potential where nearly 80% of the murine HCN channels are activated in physiological conditions (Meuth et al., 2006). Upon reaching -100 mV, the current administered was kept constant and the membrane potential was left to relax (sag) for 1.5 seconds, and the potential difference between the peak amplitude and the potential at 1.5 seconds was measured as an index of $I_h$ rectification (Fig. 3A), since this sag has been shown to reflect $I_h$ (Kanyshkova et al., 2009; McCormick and Pape, 1990; Ying et al., 2006). The $I_h$ rectification before cytokine administration (baseline period) in the control, IL-1β and IL-6 groups was 15.8 ± 0.4, 15.9 ± 1.2 and 15.2 ± 1.1 mV, respectively. A one way

ANOVA test did not show any difference among groups ($p>0.05$). After 25 minutes of exposure to the cytokines, a significant difference among the three groups was detected ($p<0.05$, one way ANOVA, Fig. 3B). The IL-1β group showed a significant decrease in the $I_h$ rectification amplitude when compared to the control group (10.2 ± 1.2 mV, n=8, and 14.4 ± 0.7 mV, n=16, respectively, $p<0.05$, HSD Tukey test). After washing the cytokines for 10 minutes, no statistical difference was detected among the three groups.

*Action potential (AP) threshold*

A ramp current ranging from 0 to 500 pA lasting 1.5 seconds was applied in order to assess AP threshold. The AP threshold was determined when the first AP occurred after the LTS burst (Fig. 4A). AP threshold were -32.3 ± 0.6 (n=22), -32.2 ± 1.4 (n=11) and -28.9 ± 1.3 mV (n=9) in the control, IL-1β and IL-6 groups, respectively, at the baseline period. A comparison among these baseline values showed no statistically significant difference ($p>0.05$, one way ANOVA). Changes in the AP threshold following cytokine application were compared as shifts from the baseline value (ΔAP threshold, Fig. 4B). After 10 minutes of exposure to the cytokines a

significant difference was found among the three groups ($p<0.05$, one way ANOVA). Further comparisons found that in the IL-1β group the AP threshold was significantly reduced when compared to the control group (-1.1 ± 0.6 mV and 1.5 ± 0.5 mV, respectively, $p<0.05$, HSD Tukey test). After 25 minutes of exposure, the difference between the IL-1β group and the control group persisted (-2.3 ± 1.3 mV and 1.7 ± 0.7 mV, respectively, $p<0.05$, HSD Tukey test). After washing the cytokines a persistent and more pronounced effect of exposure to IL-1β (-5.4 ± 1.8 mV) was observed when compared to the control group (1.9 ± 1 mV, $p<0.01$).

*Low threshold calcium spike (LTS) burst*

One of the most characteristic features of thalamic relay cells is a low threshold calcium spike (LTS) crowned with a burst of action potentials that is provoked when these cell are depolarized from a membrane potential lower than -60 mV. This LTS occurs as, at hyperpolarized membrane potentials, T-type $Ca^{2+}$ channels are deinactivated. To investigate whether cytokines have any effect over the LTS burst, a rectangular hyperpolarizing current pulse driving the membrane potential to -100 mV

was applied for 1.5 seconds, which was followed by a robust LTS burst (Fig. 5A). The number of APs in each burst was compared among the three groups. The control, IL-1β and IL-6 groups had 6.4 ± 0.3 (n=22), 5.8 ± 0.4 (n=11) and 5.5 ± 0.2 (n=9) APs per burst at the baseline period, respectively, which were not statistically different ($p>0.05$, one way ANOVA). After 25 minutes of exposure to the cytokines, the number of AP was different among groups ($p<0.05$, one way ANOVA, Fig. 5B). The number of AP in the IL-1β and IL-6 groups (5.1 ± 0.5, n=8, $p<0.01$ and 5.4 ± 0.2, n-7, $p<0.05$, respectively) was significantly smaller than that in the control group (7.1 ± 0.3, n=16, HSD Tukey test), respectively.

*Neuronal excitability*

Because the cytokines might modulate AP firing through a myriad of different mechanisms, neuronal excitability was further investigated by evoking tonic AP firing with a constant depolarizing current pulse from the resting potential in the thalamic relay cells (Fig. 6A). At the baseline period, a current amplitude at which number of APs reached about 50% of its maximum that was obtained by further increase in the

current amplitude was determined for each cell. This half maximal depolarizing current was then used to count APs in the following cytokine exposure periods (Fig. 6B). The number of APs obtained at 10, 25 and 40 (after wash) minutes were compared among the three groups. Counts of AP at the baseline period were 74 ± 8 (n=22), 75 ± 8 (n=10) and 93 ± 14 (n=9) in the control, IL-1β and IL-6 groups, respectively. A Kruskal-Wallis ANOVA test did not find a significant difference among the groups at the baseline period, 10 and 25 minutes of exposure to cytokines, or after washing the cytokines for 10 minutes (Fig. 6C).

*Computer simulation*

In order to better understand how cytokines may affect membrane elctrophysiological properties, a previously described model of thalamic relay cell was modified to more precisely fit the I-V curves obtained in this study (Fig. 7A). Of notice, to achieve a non-linear I-V curve better resembling that from a real cell, adjustments of parameters relevant to $V_{rest}$, $R_{in}$ and $I_h$ had to be performed. Those included a

considerable increase in the $K_{ir}$ channels conductance ($g_{Kir}$) and in the leak chloride permeability.

Considering the similarity of effects promoted by IL-1β and IL-6 at lower membrane potentials than $V_{rest}$ corresponding to the left side of the I-V curve, which includes more hyperpolarized $V_{rest}$, decrease in $R_{in}$ and decreased amplitudes of $I_h$ rectification, efforts were made to deduce what membrane mechanisms might be affected by those cytokines. For that purpose, three hypothetical scenarios were formulated (Table 1). The first scenario consisted of solely decreasing $I_h$ conductance ($g_{Ih}$). Before implementing that change, the model neuron was tuned to have a $V_{rest}$ of -64.5 mV, a $R_{in}$ of 200 MΩ and an $I_h$ rectification of 15.7 mV, all of which were very close to those found during the baseline period of the recorded cells. To achieve a hyperpolarization in a range between -2 mV and -5.6 mV, which correspond, respectively, to the difference of the mean of $V_{rest}$ in the IL-1β group after 25 minutes compared to its baseline and compared to the mean of the $V_{rest}$ in the control group after 25 minutes, together with a decrease in $I_h$ rectification, $g_{Ih}$ had to be decreased. The more $g_{Ih}$ decreases, however, the higher $R_{in}$ became, thus making it an impossible

explanation to what was observed *in vivo*. In the second scenario the potassium permeability of the leak current ($P_{Kleak}$) was solely altered. After a 9 times increase in $P_{Kleak}$, $V_{rest}$ hyperpolarization of -3.3 mV was obtained, along with a -5.5 mV decrease in $I_h$ rectification. $R_{in}$, however, decreased by 50%, which far exceeded the drop seen both in the IL-1β group (maximum decrease of 26%) and in the IL-6 group (maximum decrease of 34%), making a sole increase in $P_{Kleak}$ an unlikely explanation for the empirical findings. The third scenario consisted of an increase in the chloride permeability of the leak current ($P_{Clleak}$). $V_{rest}$ of -67.4 mV was obtained after a 2.1 increase in $P_{Clleak}$. Accordingly, the amplitude of $I_h$ rectification decreased by -5.6 mV. $R_{in}$, however, decreased by 63%, which again exceeded the *in vivo* findings. Since none of the three scenarios could reliably reproduce the findings in real cells, combinations of the three scenarios were tested. A fourth scenario where $P_{Kleak}$ was increased 5.3 times and $g_{Ih}$ decreased by 11% promoted a -2.9 mV hyperpolarization in $V_{rest}$, a -5.5 mV decrease in the amplitude of $I_h$ rectification and a 30% decrease in $R_{in}$ (Figs. 7A-C, Table 1), all values within the range of the findings in real cells.

Simulated LTS bursts were also very sensitive to modulation of the above mentioned conductance as well as of the T-type $Ca^{2+}$ conductance. Without modulation in the model cell, an LTS burst consisted of 6 spikes. After the above mentioned combined modulation of $P_{Kleak}$ and $g_{lh}$ mimicking the effects of IL-1β and IL-6, spike number of the LTS burst was reduced to 4 (Fig. 7D), supporting previous reports showing that the LTS burst is very sensitive to modulation of those conductance (Tscherter et al., 2011). Decreasing the T-type $Ca^{2+}$ conductance by 25% was also enough to remove two spikes from the 6 spikes LTS burst.

## Discussion

From the experiments above it is possible to conclude that IL-1β and IL-6 may affect information processing in thalamic relay cells in different ways: (1) IL-1β hyperpolarizes the resting membrane potential of thalamic relay cells, (2) near the resting potential, IL-1β and IL-6 reduce the membrane resistance, (3) IL-1β decreases the $I_h$ rectification amplitude and (4) lowers the AP threshold., and (5) IL-1β and IL-6 decrease the number of APs in the LTS burst.

### *Resting membrane potential*

The resting potential of thalamic relay cells in dLGN is dependent on synaptic input as well as on intrinsic membrane properties (Wijesinghe et al., 2013). Among the latter determinants, hyperpolarization-activated cyclic nucleotide-gated (HCN) channels and $K^+$ leak channels were demonstrated to play a counterbalancing role on it, being that the former, when up regulated, promotes depolarization, while the latter promotes hyperpolarization (Meuth et al., 2006).

Previous studies have shown that IL-1β promotes depolarization in resting potential of magno and parvocellular neurons in the hypothalamic paraventricular nucleus (Ferri and Ferguson, 2003; Ferri et al., 2005) and in magnocellular cells in the hypothalamic supraoptic nucleus (Chakfe et al., 2006), while promoting hyperpolarization in pre-optic area/anterior hypothalamus (Tabarean et al., 2006). Rather little is known about the effects of IL-6 over neuronal resting potential in the brain.

The findings of the present work demonstrate that IL-1β promotes hyperpolarization after 25 minutes of exposure. This effect may take place both through intrinsic and/or extrinsic pathways. What supports the former is the concomitant decrease of the $I_h$ rectification. This effect, however, could happen as a consequence of IL-1β action on other cells that in turn affect relay cells synaptically or humorally and promote either a decrease in the HCN conductance or an increase in the $K^+$ leak current. The interneuron network in dLGN is known to consist of GABAergic neurons, which directly modulate relay neurons through both $GABA_A$ and $GABA_B$ actions (McCormick, 1992). This extrinsic action of IL-1β by increasing inhibitory synaptic inputs cannot be

ruled out, which may rather be one of the main contributors for the hyperpolarization of resting membrane potential.

*Membrane resistance*

The membrane resistance of neurons is dependent on the population of ion channels opening at specific membrane potentials. In the present study the membrane resistance was analyzed near the resting potential, because at such potentials the membrane usually shows its lowest conductance as is evident from the I-V curve (Fig. 2B). Also, populations of ion channels, such as T-type $Ca^{2+}$ channels and HCN channels, which have a major role in computation of input information and in modulation of output, are sensitive at this range of membrane potential which overlaps with steep parts of their activation and inactivation curves (He et al., 2014; Meuth et al., 2006; Tscherter et al., 2011), thus making it a major point of interest.

IL-1β has been shown to decrease input resistance in neurons in the pre optic hypothalamus and in the basolateral amygdala (Tabarean et al., 2006; Yu and Shinnick-Gallagher, 1994). In hippocampal neuronal cultures exposed to

lipopolysaccharide (LPS), where there was increase in the IL-1β and IL-6 levels, membrane resistance was also decreased (Hellstrom et al., 2005). On the other hand, chronic exposure to IL-6 increased input resistance in Purkinje neurons (Nelson et al., 2002).

The present work has found that both IL-1β and IL-6 decrease the membrane resistance near the resting potential in relay neurons of dLGN. Again, it is not clear how these effects take place in the thalamus. However, they are in accordance with the observed hyperpolarizing effect, which can be explained by increases in $K^+$ and $Cl^-$ leak conductance by means of the computer simulation. An increase in $Cl^-$ permeability could occur as a result of increased inhibitory activity to the relay neurons through GABAergic synapses.

*HCN conductance*

HCN cation channels are encoded by the HCN gene family and have four subtypes, HCN1-4. These channels are activated upon hyperpolarization and conduct an inward and excitatory current, $I_h$, in the nervous system. In thalamic relay neurons they

depolarize and stabilize the resting membrane potential, trigger the burst mode of firing along with T-type $Ca^{2+}$ channels, and by doing so play a major role in delta rhythms and spindle oscillations (He et al., 2014; Meuth et al., 2006; Tscherter et al., 2011).

Here we found that IL-1β caused a decrease in the amplitude of $I_h$ rectification, which is attributable to HCN channels, after 25 minutes of exposure, when compared to the control group. According to the computational simulations, the decrease in amplitude of $I_h$ rectification is more likely to occur due to decreased membrane resistance in combination with decreased $I_h$ conductance, rather than by only one of these changes.

*AP threshold*

IL-1β, but not IL-6, decreased the threshold of APs in thalamic relay cells. A similar effect was found in dorsal root ganglia (DRG) of rats (Binshtok et al., 2008), being one of the possible mechanisms behind increased excitability and pain hypersensitivity to peripheral stimuli during inflammatory conditions. On the other

hand, IL-1β may inhibit $Na^+$ currents in cortical neurons (Zhou et al., 2011), thus showing a rather cell-type specific action.

Excitability of relay cells under cytokine exposure was also investigated, resulting in no significant changes in the firing rate on depolarization (Fig. 6). However, it is important to note that the firing rate of a cell is the product of a variety of factors including resting potential, membrane resistance, afterhyperpolarization currents and so on. Thus there is a possibility that the firing rate on depolarization is not a sensitive index for excitability. The lower AP threshold upon IL-1β exposure could be regarded as an indication of increased excitability.

*Glial interaction*

Astrocytes and microglia are widely known for their immunologic roles in the CNS. While the former has been demonstrated to perform phagocytosis (Bechmann and Nitsch, 1997), respond to inflammatory mediators, such as IL-1, and secrete IL-6 and TNF-α (Lee et al., 1993), the latter has been related to apoptotic cell clearance (Takahashi et al., 2005), and, when activated, to the release of a miriad of inflammatory

mediators, including cytokines, chemokines and complement proteins, among other factors (Czirr and Wyss-Coray, 2012; Hanisch and Kettenmann, 2007).

But besides their house keeping functions, those cells also have important regulatory roles in synaptic transmission and in brain rythms. In the thalamus, astrocytes regulate GABAergic and glutamatergic transmission (Beenhakker and Huguenard, 2010; Pirttimaki and Parri, 2012) and play a role in the regulation of sleep rythms (Halassa et al., 2010), and their disfunction may be related to pathologic conditions such as epilepsy (Amiri et al., 2012). Microglia has also been demonstrated to act in proper establishment of synaptic transmission in the thalamus (Hoshiko et al., 2012). Considering the responsiveness to neuro-immune signals, as well as the importance of those two cell types in the thalamus, it is possible that some of the effects of the cytokines here investigated could take place through a glial-neuron interaction. Future investigation should address that hypothesis in order to provide deeper insights on it.

*LTS burst*

Being one of the most characteristic electrophysiological signatures of thalamic relay cells LTS bursts occur mostly as a combination of the activities of T-type $Ca^{2+}$ channels and HCN channels. Molecular cloning and expression studies have established the existence of three genes encoding the Cav3.1, 2 and 3 isoforms of T channels with multiple splice variants (Catterall et al., 2003; Perez-Reyes, 2003), which permit a variety of shapes of $Ca^{2+}$ currents. Previous studies have shown that even very small modifications in the currents that constitute LTS bursts may have an impact on the pattern of bursts in thalamic relay neurons (Tscherter et al., 2011). As the T-type $Ca^{2+}$ channels are targets of modulation by a variety of neurotransmitters and hormones, cytokines might also affect them.

The present study found a reduction in the number of AP in LTS bursts after 25 minutes of exposure to IL-1β and IL-6 (Fig. 5B), which could be attributed to both decreased membrane resistance and decreased HCN conductance, as reproduced in the computer simulation. A modulatory action over T-type $Ca^{2+}$ channels, however, cannot be excluded, and further investigation is necessary to address this possibility.

*Physiopathological considerations*

In recent years immunotherapies with agents like anti-IL-5 antibody for asthma and other eosinophilic conditions, anti-IL-6 antibody for rheumatoid arthritis and anti-IL-17 antibody for ankylosing spondylitis (Alten, 2011; Baeten et al., 2013; Corren, 2011) are appearing as alternatives to the treatments of those conditions. In the same way, understanding roles played by cytokines in the brain may give rise to new and vast modalities of treatment for conditions as diverse as major depression and acute delirium.

The thalamus, being a key structure involved in sensory information processing and relaying, has already been implicated in conditions where a faithful transmission of signals to the cortex appears to be impaired, like schizophrenia and other syndromes accompanied by hallucination (Andreasen, 1997; Cronenwett and Csernansky, 2010; Schmahmann, 2003). Being a structure also intimately related to levels of consciousness, dysfunctions on it are also associated to sleep disorders and epilepsy (David et al., 2013; Keller et al., 2014). Understanding whether and how those conditions are related to inflammatory processes is of major importance for the

development of new effective therapeutic strategies.

*Final considerations*

The main purpose of the present study was to investigate whether IL-1β and IL-6 have any action over electrophysiological properties in relay neurons of dLGN. Since it is the first time that this relation is investigated, any finding may be considered relevant.

Thalamic relay neurons, possessing so many particular electrophysiological characteristics, offer a very good model to study the influence of cytokines over ionic conductance, and at this point it is possible to conclude that IL-1β and IL-6 appear to exert influence on dLGN relay neurons of the thalamus in different manners. Still, more work has to be done for better elucidating these actions.

**Legend to figures**

**Figure 1.** Normalized resting potential ($V_{rest}$). A, Time course of resting potential changes in each cytokine group indicated as difference from the baseline $V_{rest}$ (see Results, $\Delta V_{rest}$, *p<0.05, one way ANOVA). B, $\Delta V_{rest}$ after 25 minutes of exposure to cytokines, where a significant difference was found. *p<0.05, HSD Tukey test.

**Figure 2.** Input resistance ($R_{in}$). A, Voltage traces obtained from a thalamic relay cell that was not subjected to a cytokine with injected rectangular current pulses of step-wise increments. The dotted lines refer to the points from where the I-V curve was taken. B, A representative I-V curve obtained from the experiment shown in A. The range from where $R_{in}$ was measured is indicated. C, Normalized $R_{in}$ changes throughout the experiment (*p<0.05, one way ANOVA).

**Figure 3.** Inward rectification attributable to $I_h$ ($I_h$ rectification) at -100 mV. A, Typical example of $I_h$ rectification (arrow) at the baseline period (black line) and after

administration of IL-1β (gray line). Inset: square current pulse applied. B, Time course of $I_h$ rectification throughout the experiment. Data are shown as the means of the $I_h$ rectification amplitudes (*$p<0.05$, one way ANOVA).

**Figure 4.** Action potential (AP) threshold. A, APs evoked with a ramp current. Relay cells showed an LTS burst (asterisks) followed by APs with an increasing injected current. AP threshold was taken from the membrane potential where the initial AP started (arrows). The gray and black traces were taken at the baseline period and after 25 minutes of exposure to IL-1β, respectively, in the same cell. B, Time course of the changes of AP threshold (ΔAP) throughout the experiment in the three experimental groups (*$p<0.05$, one way ANOVA).

**Figure 5.** Characteristics of APs in the LTS burst. A, An LTS burst seen just after a hyperpolarizing current pulse termination. Insets indicate either expanded LTS burst or the hyperpolarizing current pulse shape. B, Time course of the number of APs in LTS bursts throughout the experiment (**$p<0.01$, one way ANOVA).

**Figure 6.** Number of APs evoked in tonic mode. A, Representative thalamic relay neuron firing in tonic mode. B, Number of APs evoked by rectangular current pulses of different amplitudes at the baseline period. The current amplitude that triggered 50% of the maximum number of APs (dotted line) at the baseline was applied throughout the experiment. C, Time course of mean of number of APs throughout the experiment.

**Figure 7.** Computer simulations. A, I-V curves. Ionic conductance in the model neuron were adjusted (dashed line) in order to better resemble an I-V curve obtained from a real thalamic relay cell (black line). The dotted line shows an I-V curve simulated with not adjusted parameters including $g_{Kir}$ (refer to Results). B, Simulated membrane potential changes by a rectangular hyperpolarizing pulse. The black line shows simulated membrane potential after adjustments of parameters for the I-V curve to better fit real data at the baseline period. The gray line represents a simulated membrane potential change after decreasing $g_{Ih}$ and increasing $P_{Kleak}$ in the former model, which corresponded to the findings observed in the cells exposed to IL-1β or IL-6. C and D,

Magnified views of B. C shows the $I_h$ rectification with an arrow pointing to -100 mV.

D, The LTS burst shapes.

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Table 1. S*imulation settings*

| Simulated setting | Real representative cell at baseline | Model cell before interventions | 1st scenario (Decreased $g_{Ih}$ ) | 2nd scenario (Increased $P_{Kleak}$ ) | 3rd scenario (Increased $P_{Clleak}$ ) | 4th scenario* (Increased $P_{kleak}$ and decreased $g_{Ih}$ ) | Means obtained from cells exposed to IL-1β |
|---|---|---|---|---|---|---|---|
| $V_{rest}$ (mV) | -65.1 | -64.5 | -66.5 | -67.8 | -67.4 | -67.4 | -66.4 |
| $I_h$ rectification (mV) | 15.9 | 15.7 | 10.3 | 10.2 | 10.3 | 10.2 | 10.3 |
| $R_{in}$ (MΩ) | 199 | 200 | 318 | 100 | 75 | 140 | 155 |

* Only when increased $P_{kleak}$ was combined with decreased $g_{Ih}$ it was possible to obtain values of $V_{rest}$, $I_h$, and $R_{in}$ compatibles with those found in real cells.

Fig.1 – Samios and Inoue

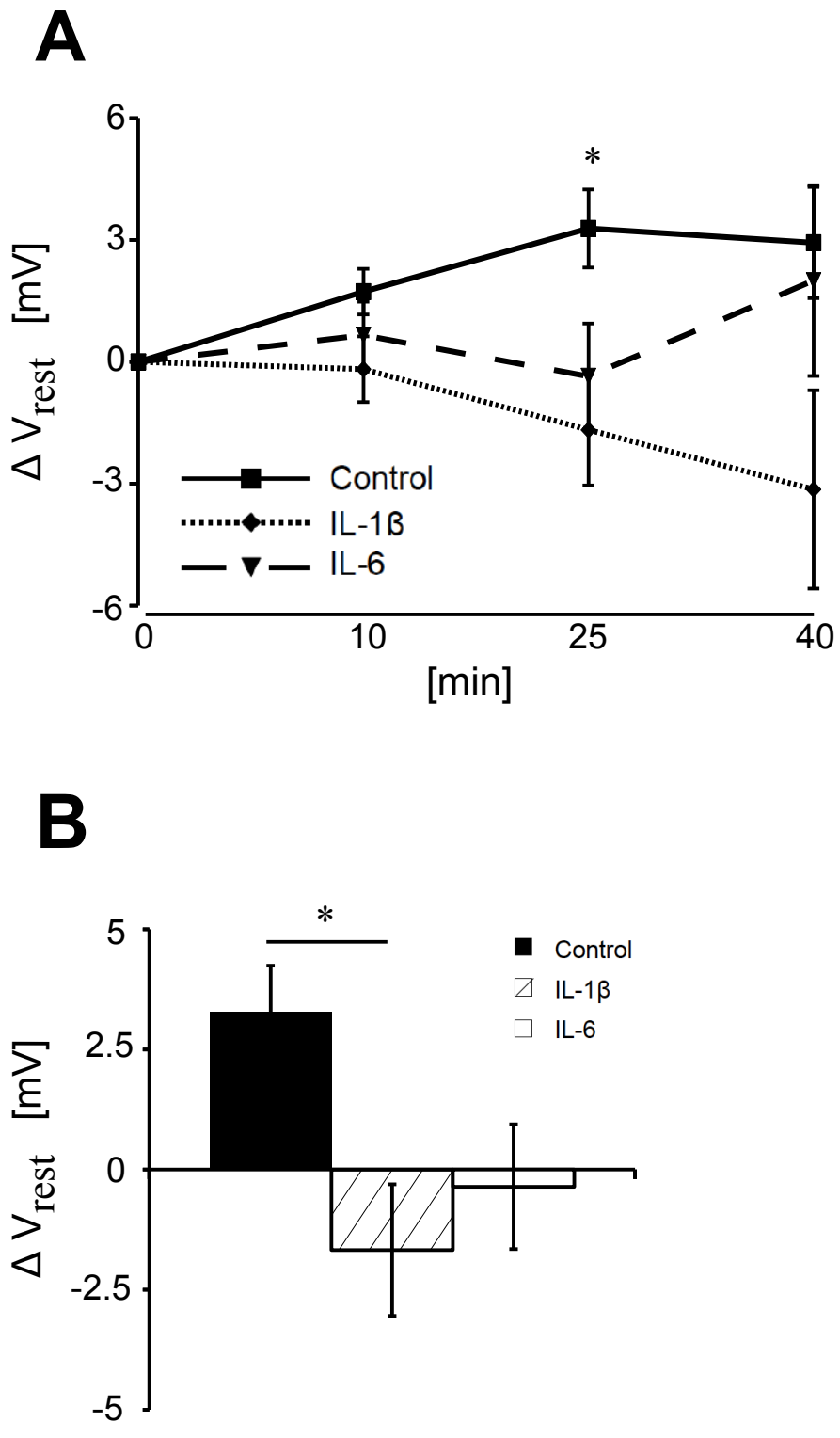


Fig. 2 – Samios and Inoue

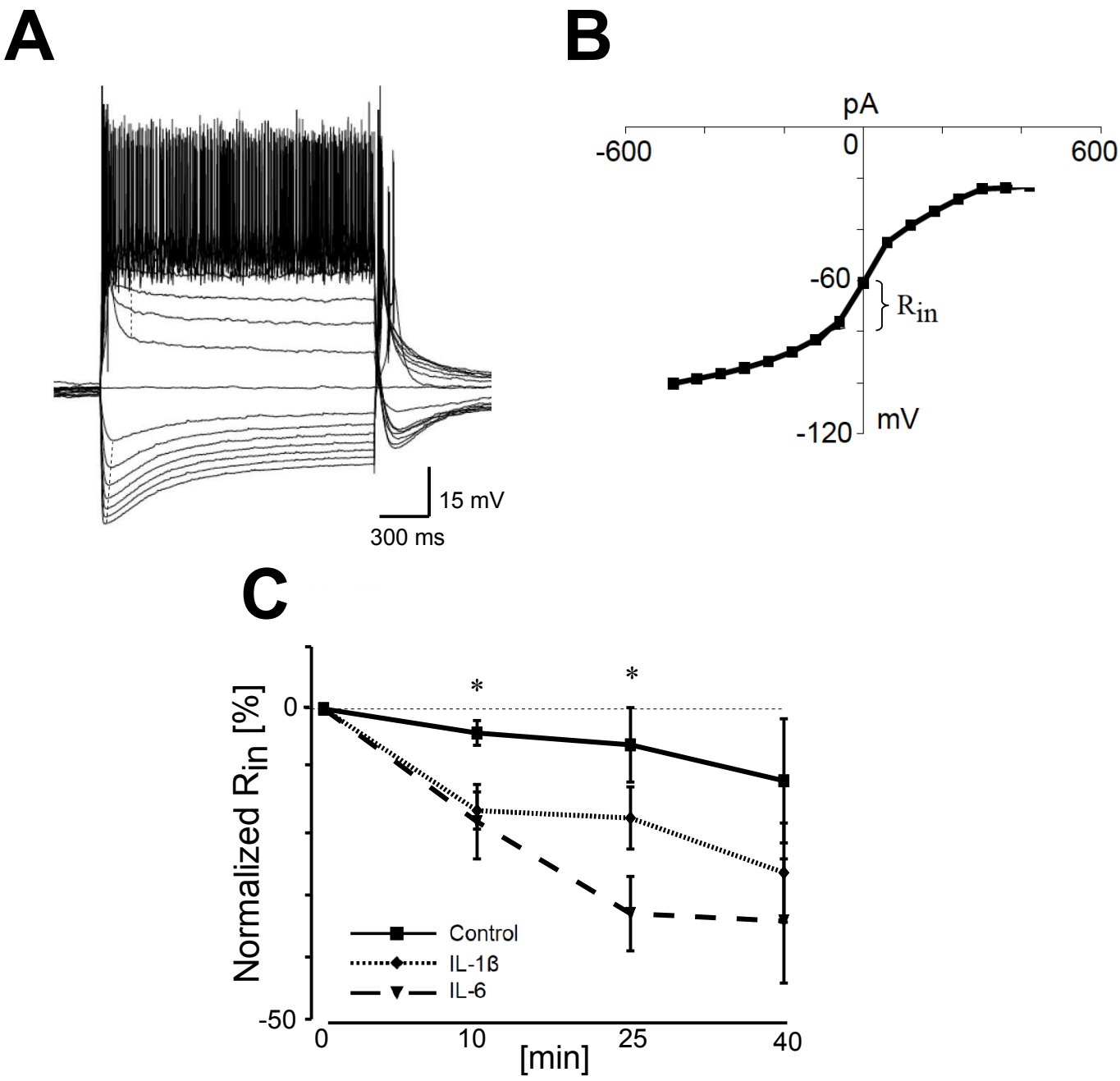


Fig. 3 – Samios and Inoue

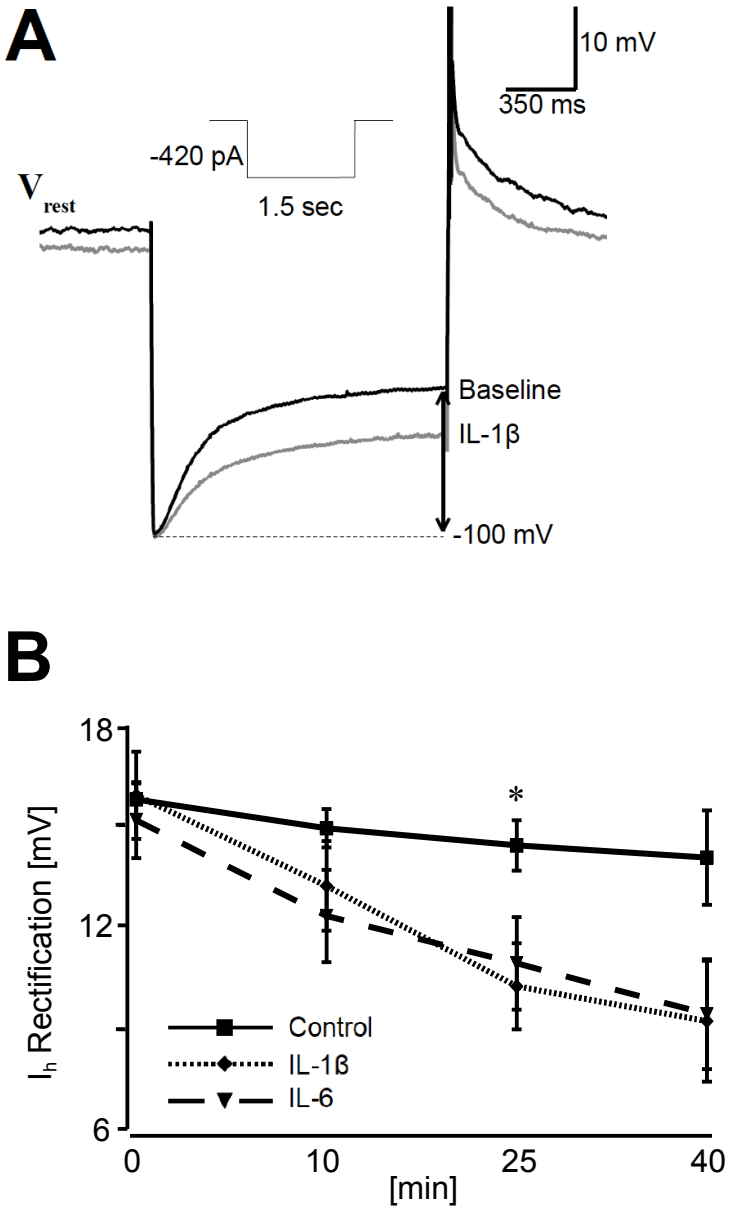


Fig. 4 – Samios and Inoue

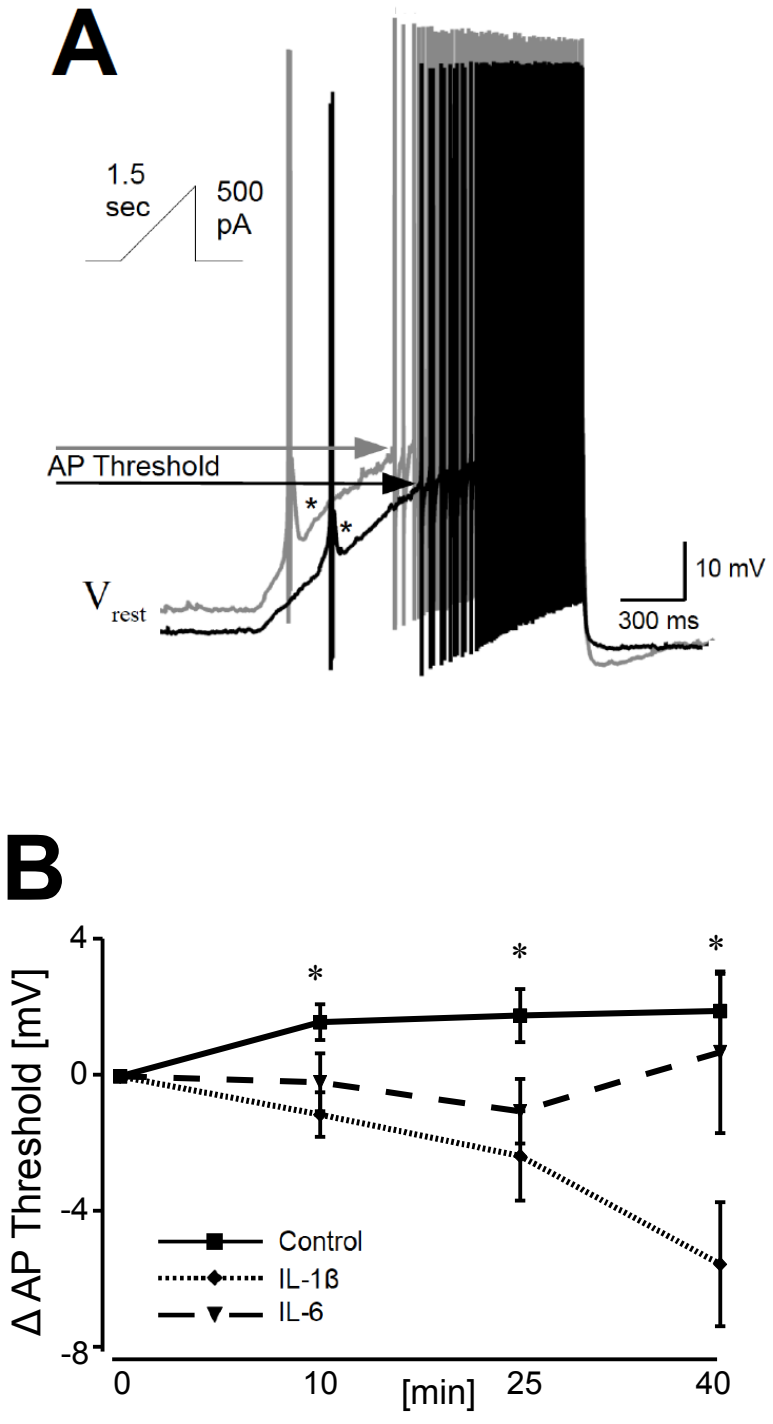
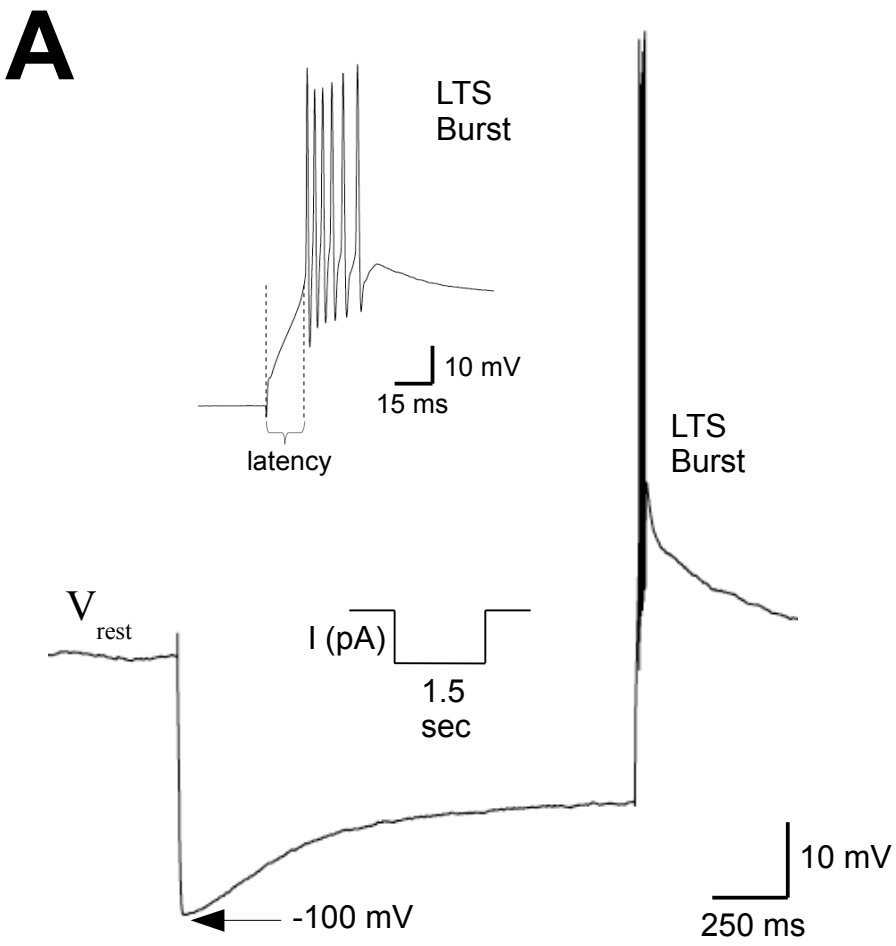


Fig. 5 – Samios and Inoue



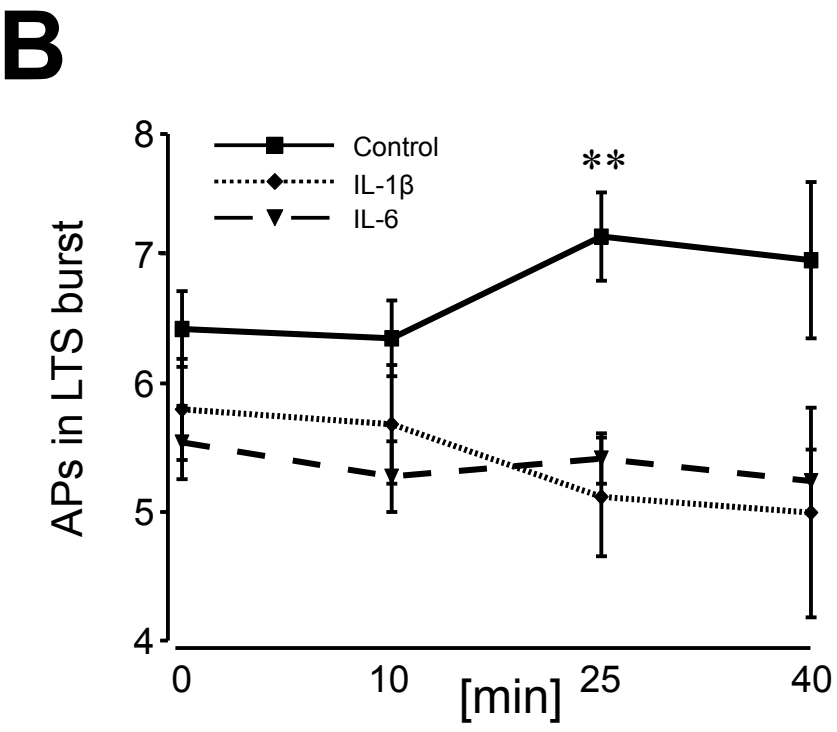



Fig. 6 – Samios and Inoue

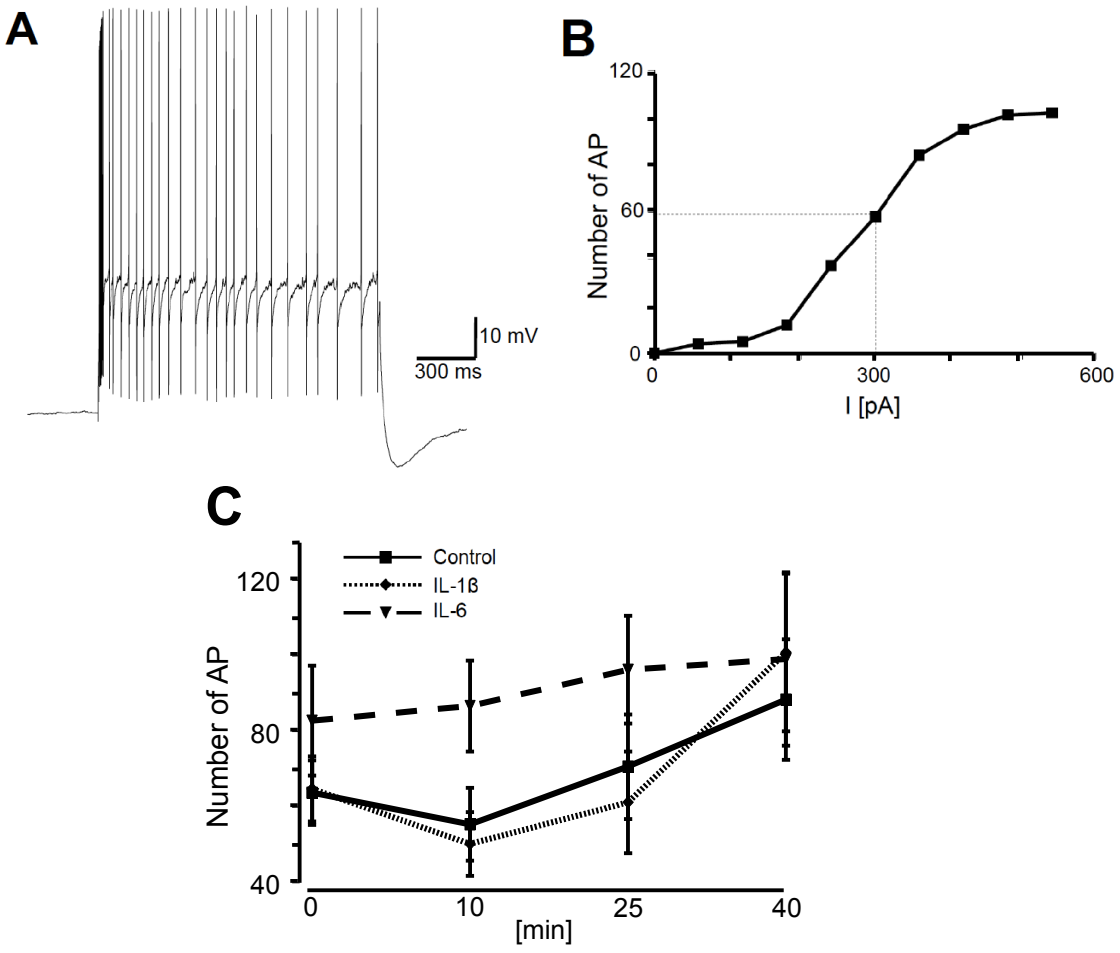


Fig. 7 – Samios and Inoue