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Original contribution

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Effects of estradiol or progesterone on principal cells from amygdala complex evaluated *in silico*

Efectos del estradiol y la progesterona sobre las células principales del complejo amigdalino evaluados *in silico*

Abstract

Introduction: Amygdala neuronal responses play a key role in fear conducts. Principal Neuron (PN) is one of the most important generators of the output in the amygdala neuronal network. PN has excitatory connections to other PN and receives inhibitory synapsis from GABAergic interneurons acting on gGABAA, also receives thalamic and cortical inputs which activate postsynaptic gNMDA, gAMPA and gL (L-type Ca2+ channels). It has been proposed that main neurotransmission in amygdala could be modulated by estradiol (ES) and/or progesterone (PRG).

Objetive: To implement a deterministic PN model with reported membrane conductances and to evaluate discharge changes induced by ES and/or PRG.

Methods: Seven different scenarios were tested: a) Control conditions running unchanged membrane ion and synaptic currents; b) 20% reduction in GABAA current for ES effect; c) 20% increase in AMPA and NMDA currents for ES effect; d) b + c; e) 20% reduction AMPA/Kainate for PRG effects; f) 20% increase GABAA current for PRG effects; and, g) e + f.

Results: ES shows a strong excitatory effect more dependent on gGABAA reduction associated with a long lasting increase of gAMPA than for the increase on gAMPA and gNMDA when evaluated separately, however, a combination of these factors, which are the actual situation, shows a more intense and lasting neuronal excitation. PRG shows a strong inhibitory effect avoiding any discharge that was more depended to the fast increase and long lasting effect on gGABAA than due to decrease in gAMPA and gNMDA. Combination of these factors shows no synergic, not even additive inhibitory effects.

Conclusion: These results strongly support the notion that ES and/or PRG participate on amygdala principal neuronal responses involved in fear, anxiety and nocifensive behavior probably associated to gender.

Keywords

Amygdala, principal neuron, GABAA, AMPA, NMDA, estradiol, progesterone.

Resumen

Introducción: Las respuestas neuronales de la amígdala juegan un papel clave en las conductas del miedo. La neurona principal (PN) es uno de los generadores más importantes de la salida en la red neuronal del complejo amígdalino. PN tiene conexiones excitatorias a otras PN y recibe sinapsis inhibitoria de interneuronas GABAérgicas que actúan via gGABAA, además recibe entradas talámicas y corticales que activan gNMDA, gAMPA y gL (canales de Ca+2 tipo L) postsinápticas. Se cree que el estradiol (ES) y/o la progesterona (PRG) modulan la neurotransmisión del complejo amígdalino.

Objetivo: Se implementó un modelo deterministico de PN con conductancias de membrana conocidas y se probaron cambios en el patrón de descarga neuronal inducidos por ES y/o PRG.

Métodos: Se probaron siete escenarios diferentes: a) Condiciones controles sin cambios en los flujos iónicos de membrana ni en las corrientes sinápticas; b) Reducción del 20% de la gGABAA como efecto del ES; c) Aumento del 20% de las gAMPA y gNMDA como efecto del ES; d) b + c; e) Reducción del 20% de gAMPA / gKainate como efecto de la PRG; f) Aumento del 20% de la gGABAA como efecto de la PRG; y g) e + f.

Resultados: El ES mostró un fuerte efecto excitatorio más dependiente de la reducción de la gGABAA asociada con un aumento duradero de gAMPA, que debido al aumento en gAMPA y gNMDA evaluados separadamente, sin embargo, la combinación de estos factores, que es una situación mas real, muestra una excitación neuronal mas intensa y duradera. La PRG muestra un fuerte efecto inhibitorio evitando cualquier descarga neuronal, lo cual fue más dependiente del rápido aumento y la larga duración de gGABAA que por la disminución de gAMPA y gNMDA. La combinación de estos factores no muestra efectos inhibidores sinérgicos, ni aditivos.

Conclusión: Estos resultados apoyan firmemente la noción que el ES y/o la PRG participan en las respuestas de las neuronas principales de la amygdala implicadas en el miedo, la ansiedad y el comportamiento ante un estímulo nocivo, siendo probablemente asociado al género.

Palabras clave

Amígdala, neurona principal, GABAA, AMPA, NMDA, estradiol, progesterona.

Introduction

A threat induces a behavioral and emotional reaction which derives in fear, anxiety and an initial increased pain response. Sensory input induces a thalamus activation with a multifactorial response for survival behavior, being the amygdala the most important trigger for the increased cortical arousal associated to fear.[1] This process includes physiological changes such as increased heart and respiratory rate, blood pressure, stress hormone release and defensive/attack behavior.[2–4]

Thalamic inputs send information to the lateral amygdala from where it is projected to the basal nucleus (BA) and intercalated cells (IC), these cells send GABAergic projections to central medial nucleus while BA conducts excitatory inputs to IC and to medial sector neurons of central amigdala (CeM). Additionally, the infralimbic cortex returns transmissions to IC and CeM cells which project to brainstem structures mediating fear responses.[4–6] Different studies support the notion that principal neurons (PN) of the BA and Interneurons (IN) are the most important generators of the output in the amygdala neuronal network.[1,5,6,8]

PN shows different Ca2+-dependent K+ current which induces its typical spike frequency adaptation to an injected depolarizing current.[7] This neuron has excitatory connections to other PN and receives inhibitory synapsis from GABAergic interneurons.[7–9] PN also receives thalamic and cortical inputs which activate postsynaptic gNMDA and gL (L-type Ca2+ channels) to increase Ca2+ ions influx.[10–12] Conditioned fear can be inhibited by reducing this gNMDA denoting its relevant role such as neuronal substrate for fear conditioning.[6]

Main excitatory and inhibitory neurotransmission in the amygdala has been proposed to be modulated by estradiol (ES) and progesterone (PRG),[13] in fact, both, glutamate (AMPA and NMDA) and γ-amino-butyric acid (GABAA and GABAB) membrane receptors show strong responses to ES or/and PRG with possibly contrasting roles in many encephalic areas.[13] PRG intensely reduces glutamate response via attenuation of AMPA and kainate receptors in a proportionally dose-dependent way and enhance GABAA responses.[14] In contrast, ES increases AMPA/Kainate and NMDA responses and reduces GABAA conductance.[13]

There are evidences that, in the amygdala, ES and PRG may influence anxiety, fear, and pain behaviors, interacting not only via classical nuclear receptor (genomic action), but also via non genomic action by membrane associated receptors such mER correlated to ERK, cAMP/PKA, PLC/PKA, Ras/Raf/MAPK and PI3K/Akt signaling pathways for ES and mPR, PGMRC, ERK, cAMP/PKA, PKG, Ca2+ influx/PKC, PI3K/Akt signaling pathways for PRG.[13]

The effect of gender, expressed by the presence, absence or physiological cyclic variation of different sex hormones on amygdala neurons is not clearly understood.[13,15] The implication of a possible differentiation but also, cyclic mood variations, could offer new insight about physiological, physiopathological and psychological situations such as anxiety disorders which are more frequent in women than men.[13,16,17] Clinical evidences show that females suffer irritable bowel syndrome more frequently than males, but also strongly associated to the ovarian hormone cycle, amygdala activation and pain response.[18] Hormonal implants within the amygdala showed in animal models that progesterone and/or estradiol increases visceral

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pain responses but not somatic sensation,[18] and that bilateral application of corticosterone at the central amygdala induces visceral hypersensitivity, anxiety-like behavior and somatic allodynia. In contrast, systemic or intra-amygdala administration of ES and/or PRG increased open field central entries and open arm time in the plus-maze, decreased freezing postshock time and increased latencies to lick paws in conductual tests. On the other hand, a brief hyperpolarization and increased potassium conductance were reported to be induced by [17]β-Estradiol.[19] These facts suggest that ES and PRG have contrasting effects in the amygdala to modify anxiety, fear, and/or pain responses.[20]

Different computational models have provided clear evidence about interaction of specific neuronal types within the amygdala complex network involved in the fear-conditioning mechanism, these models give insight about inhibitory and/or excitatory responses which match to in vivo results about synapses, chemical mediators and cellular responses.[4,21–23]

Despite the well known presence of both glutamate (AMPA and NMDA) and λ-amino butyric acid (GABAA and GABAB) neurotransmission in the amygdala complex and that they could be modulated by ES and/or PRG, the balance and contribution of each of them to the final PN discharges pattern is less understood. Thus, the present study aimed to *in silico* analysis of how ES and/or PRG modulates discharge patterns in a single-cell model of PN from the amygdala complex.

Methods

This study was approved by the institutional bioethics committee (Dirección de Investigación y Producción Intelectual, FCS, Universidad de Carabobo). Freely available CClamp neural simulation program;[24] www.EOTNprogram.org was used, running on an I5 like CPU computer. This program written in Turbo C++, allows simulations of neuronal discharges in current clamp technique using cell models with user defined ionic currents in a membrane that behaves uniformly and simultaneously.[24]

Principal Neuron (PN) Model

PN was deterministically modeled as one compartment represented by a soma of 25μm diameter and a total membrane area of 1960μm2. Membrane parameters were membrane capacitance (Cm) = 2μF.cm-2, Membrane longitudinal resistance (Rm) = 55KΩ.cm-2, and Membrane access resistance (Ra) = 175 Ω.cm-1. Leakage reversal potential was set to -67 mV. The resulting resting potential (Vrest) was -69.5 mV, input resistance was ~150MΩ, and the membrane time constant (τm) was 30 msec. The model includes the following conductances (g in nS units): fast transient sodium gNa=18; potassium delayed rectifier gK+=0.9; fast and transient depolarization-activated potassium gA=1.35; depolarization activated persistent muscarinic potassium gM=0.0001, high threshold voltage activated calcium Ca++ gL=50, low threshold Ca++ current gT=10 and a slow voltage-independent afterhyperpolarization gAHP=0.0001; Na+ leak=0.054 and K+ leak=1 as previously reported.[4,7,9,21–23,25] Synaptic parameters were defined by inhibitory postsynaptic potential (IPSP)=0.05nA; IPSP kinetic=0.3; GABAA weight 0.7; GABAB=0; IEPSP=0.1nA; and excitatory postsynaptic potential (EPSP) kinetics=0.5, AMPA weight=1; NMDA weight=1.

Background neuronal activity of PN model was adjusted by IPSP and EPSP input frequency to achieve a single spike at a low firing rate of 0.7 Hz according to in vivo recording but also *in silico* model of PN.[9,21] The simulation model includes the following intra- and extracellular ionic concentrations (mM): [Mg++]o=1; [Ca++]o=2; [Ca++]i=10nM; [K+]o=3.1; [K+]i=135; [Na+]o=145; [Na+]i=31; [Cl-]o=120; [Cl-]i=7. All scenarios were run at 35oC well within the temperature range of experimentally reported parameters used in the present study.

Model Validation

PN model was *in silico* tested by the intracellular injection of a +2 nA despolarizing current during 500 ms. The evaluation of the resulting discharge patterns match those reported by in vivo experiment.[21]

Experimental Protocol

Effects of ES or PRG on the discharge patterns of PN were tested in 7 different scenarios: a) Control conditions running unchanged all membrane ion and synaptic currents (see PN Model of this section); b) 20% reduction in GABAA current (from weight 0.7 to) for ES effect; c) 20% increase in AMPA and NMDA currents for ES effect; d) b + c; e) 20% reduction AMPA/Kainate for PRG effects; f) 20% increase GABAA current for PRG effects; and g) e + f

Results

The implemented PN model reproduced in vivo electrophysiological characteristics of this cell type with typical low frequency spontaneous discharge patters, even during the injection of intracellular positive (+2 nA) current, the PN responds with depolarization and action potentials sequence with spike frequency adaptation and an afterhyperpolarization (Fig.1), while negative current (-2 nA) induced a stable hyperpolarization during whole injection phase and a rebound despolarization due to low threshold Ca++ (gT) currents (Figure 1).

Single spike control conditions

Basal conductance of AMPA, NMDA and GABAA and defined synaptic currents of the PN model generates a single spike with a 3 ms bimodal ascending phase and a long (46ms) repolarization phase (Figure 2). A constant gAMPA was observed along the recording with a low long lasting (>30ms) increase up to 8nS in gNMDA but with a 6ms lag from the beginning of the spike depolarization (Figure 2). On the other hand, a transient gGABAA increased up to 40nS with a long lasting (>50ms) decay (Figure 2).

ES effects

Estradiol, implemented in the PN model by a 20% reduction on gGABAA induced a double spike discharge, the first one similar to the control, the second, smaller and wider with a long lasting repolarization (Figure 2). The ascending phase of the

Figure 1. Discharge pattern of modeled PN neuron (upper panel), spontaneous first spike followed by a burst discharge induced by a depolarization current injection (+2nA) showing a typical frequency adapting response. Over plotted is the hyperpolarization response to a negative current injection (-2 nA). The lower panel shows the over plotted current injections.

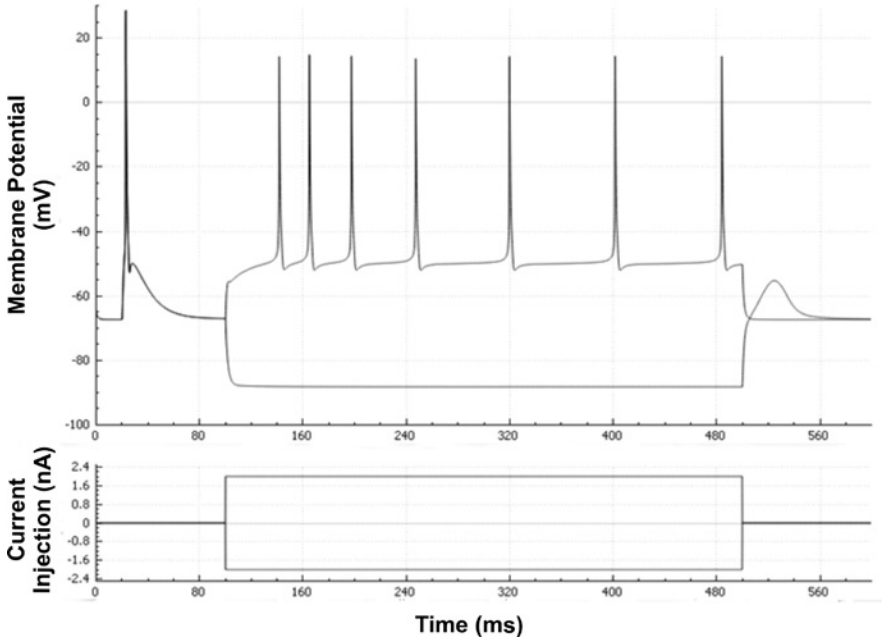
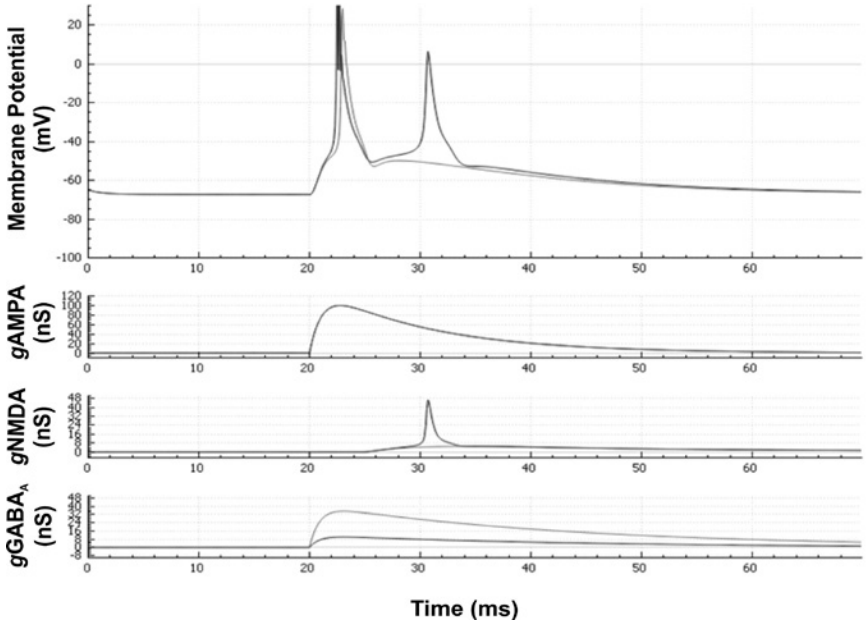


Figure 2. Single discharge of modeled PN neuron (mV) and AMPA, NMDA and GABAA conductances (g) respectively obtained during control conditions (gray lines) and estradiol scenario (dark lines) characterized by a 20% reduction of gGABAA.



first spike shows an increase up to 100nS of gAMPA for at least 40ms. Slow increase of gNMDA 6ms after the gAMPA activation preceded its 40nS peak at 31ms after the first spike depolarization (Figure 2). The gGABAA under ES effects was characterized by a stark increase up to 40nS with the same long lasting decay observed at control conditions (Figure 2). The ES effects evaluated by a 20% increase on gAMPA and gNMDA did not induce significant changes of discharge patterns to those observed with 20% reduction on gGABAA (Figure 3). Combination of 20% decrease of gGABAA and 20% increase of gAMPA and gNMDA were tested and resulted on damped discharge with steady stage depolarization at -30mV (Figure 4) probably induced by a damped increase in gNMDA which remains high (Figure 4) with a very long decay (>40ms).

PRG effects

Increased gGABAA (+20%) implemented as PRG effect results in no discharge pattern, only a EPSP with similar time course to control conditions with fast increase and long lasting decay for AMPA and GABAA but with progressive increase and long lasting gNMDA increase (Figure 5). Reduction in gAMPA and gNMDA by a 20% did not change this response pattern (Figure 6), neither the combination of 20% increase in gGABAA nor a 20% decrease of gAMPA and gNMDA were able to modify the discharge pattern (Figure 7).

Discussion

The present *in silico* study evaluated the discharge and excitability of a deterministic PN model from the amygdala complex to differential and combined membrane conductance changes induced by ES and/or PRG as reported previously.

We found that ES shows a strong excitatory effect more dependent on gGABAA reduction associated with a long-lasting increase of gAMPA than for the increase on gAMPA and gNMDA in separate scenarios.

Combining both factors e.g., reduction on gGABAA with increase on gAMPA and gNMDA in a near-real situation shows greater intensity and persistence of neuronal excitation. PRG shows a strong

Figure 3. Single discharge of modeled PN neuron (mV) and AMPA, NMDA and GABAA conductances (g) during estradiol scenario characterized by a 20% increase of gAMPA and gNMDA. Dark lines: control trace; gray lines: response to estradiol.

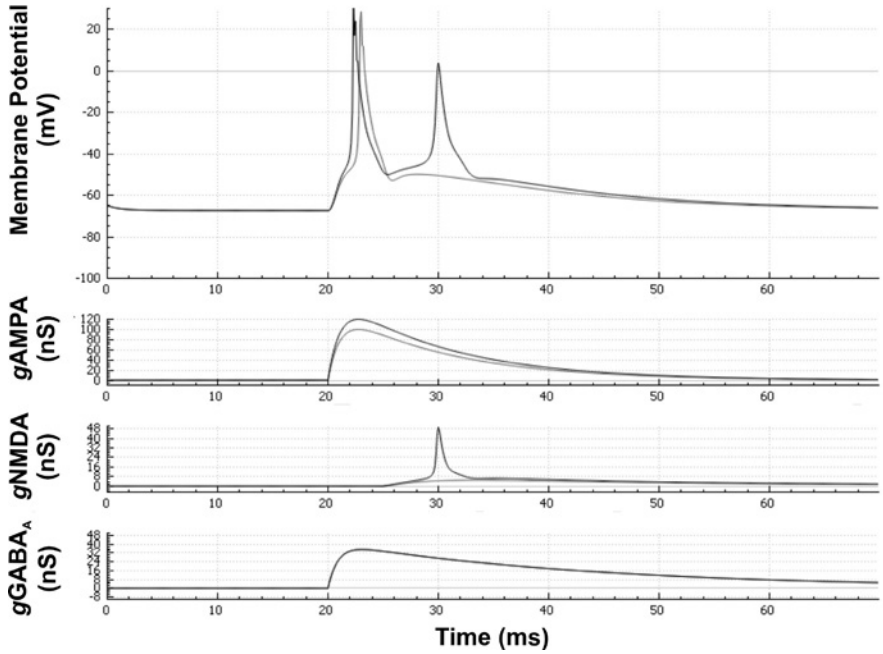


Figure 4. Single discharge of modeled PN neuron (mV) and AMPA, NMDA and GABAA conductances (g) during estradiol scenario characterized by the combination of 20% decrease in gGABAA and a 20% increase of gAMPA and gNMDA. Dark lines: control trace; gray lines: response to estradiol.

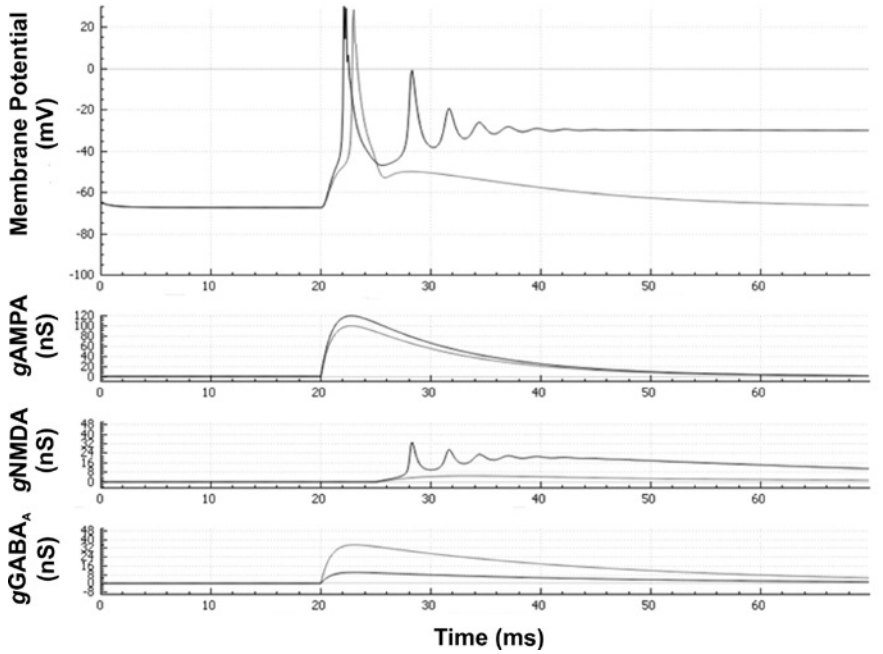
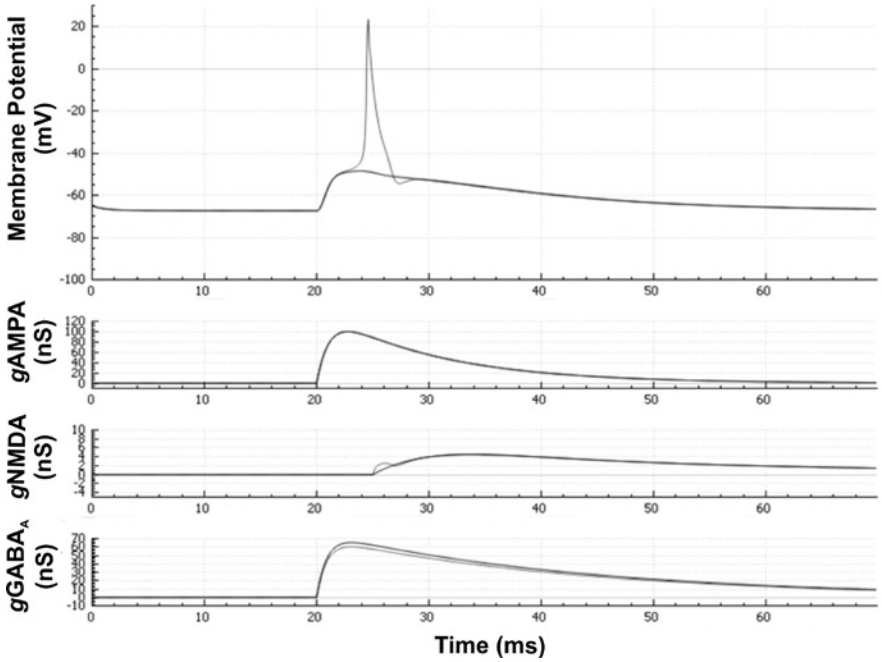


Figure 5. Single discharge of modeled PN neuron (mV) and AMPA, NMDA and GABAA conductances (g) during progesterone scenario characterized by a 20% increase of in gGABAA. Dark lines: control trace; gray lines: response to estradiol.



inhibitory effect avoiding any discharge which was more dependent on the fast increase and long lasting decay of gGABAA than due to decrease in gAMPA and gNMDA. The combination of these factors displays no synergic, not even additive inhibitory effects. The fact that implemented changes in both gGABAA and glutamate gNMDA and gAMPA were always the same e.g., 20% for each conductance strongly suggests that the main effect of both ES and PRG is exerted by changing gGABAA, decreasing or increasing it respectively.

Our *in silico* results agree to report that PRG decreases glutamate excitation[26] and increases GABA inhibition via GABAA receptors.[27] ES shows increase of the glutamate excitation via NMDA receptors[28] and decrease in GABA inhibition.[29] The ES effect on NMDA receptors has been associated with synaptic plasticity, learning and memory.[30]

Neurosteroids have been reported to act on variety types of ionotropic, i.e., voltage-gated ion channels, GABAA and NMDA, but also on serotonin and dopamine metabotropic receptors.[30,31] This type of modulation is essentially genomic because it depends on the modulation of the synthesis of receptor's protein subunits which takes from minutes to hours. On the other hand, the non-genomic action is far faster i.e., in the range of milliseconds to seconds, been associated with direct membrane receptors modulations.[32]

Both ES and PRG actions impact synaptic physiology through the activation of multiple intracellular signaling pathways[19,33] including the MAPK/ERK and the Akt pathways which are a non-genomic signaling cascade.[34] A membrane progesterone-binding protein named 7TMPR has been found to mediate fast non-genomic actions via second-messengers on presynaptic NMDA and GABAA receptors.[35] A very interesting notion is that genomic and nongenomic actions of neurosteroids could be correlated.[36] These combined effects have been also reported in the central nervous system for other classical intracellular receptor acting hormones such as thyroid hormones.[37]

Our findings suggest that ES and PRG could

Figure 6. Single discharge of modeled PN neuron (mV) and AMPA, NMDA and GABAA conductances (g) during progesterone scenario characterized by a 20% decrease in gAMPA and gNMDA. Dark lines: control trace; gray lines: response to estradiol.

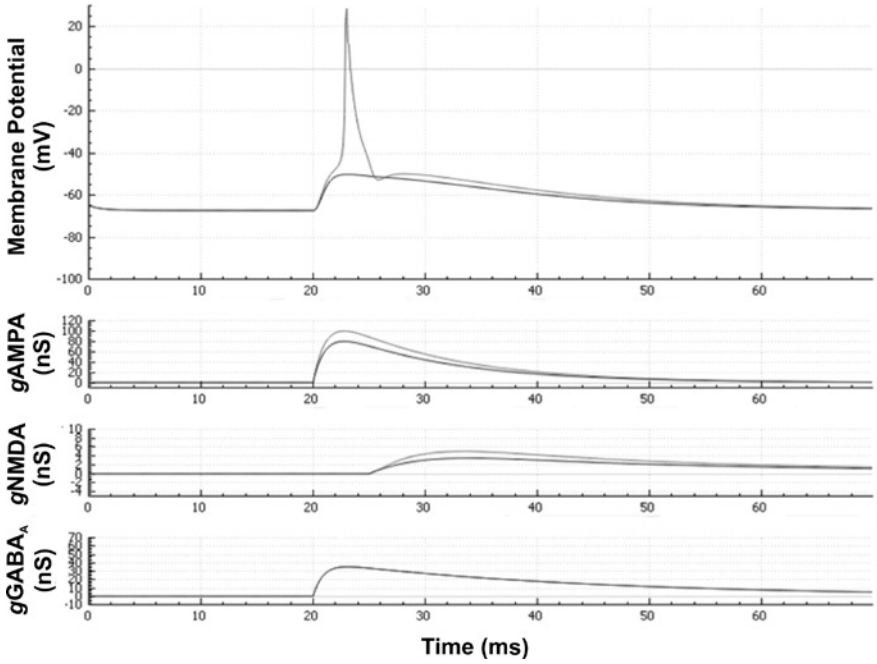
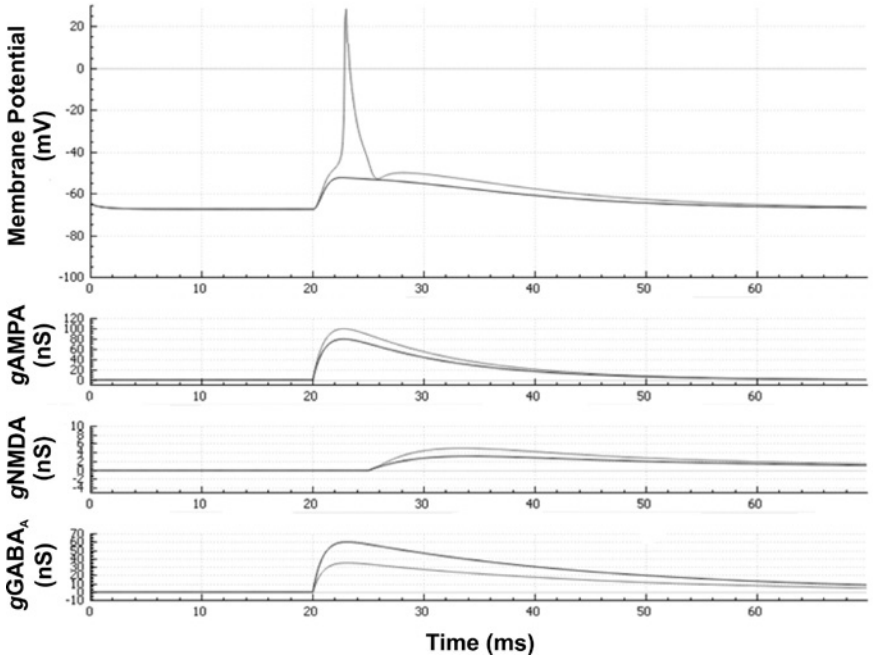


Figure 7. Single discharge of modeled PN neuron (mV) and AMPA, NMDA and GABAA conductances (g) during estradiol scenario characterized by the combination of 20% increase in gGABAA and a 20% decrease of gAMPA and gNMDA. Dark lines: control trace; gray lines: response to estradiol.



be involved in female psychological menstrual variation. The model with only ES is equivalent to the follicular phase characterized by high ES concentration, eliciting electrical excitation and subsequently the increment of neuronal response at the behavioral level. The ES+PRG simulation scenario, equivalent to the postovulatory phase, denotes the attenuating effect of PRG on ES action. This argument could be applied to gender differences response to threat, however ES levels were associated to human fear responses with similar extinction recall between men and high-estradiol women, but low-estradiol women were associated to impaired extinction consolidation.[38]

In line with these findings, animal and human studies reported that ES exerts proconvulsant activity[39,40] but shows a positive correlation between fear extinction memory consolidation and high ES levels,[41] during extinction behaviors, women in the follicular-midcycle menstrual phase have better extinction retention than women in the early follicular menstrual phase.[42]

The PRG predominance was compared in our model with the luteal phase, despite combining both hormones, the predominant effect was an increased inhibition of neuronal activity. The progressive increment of PRG absolute concentrations in the course of a normal pregnancy going together with high ES levels even though differing by one order of magnitude. High PRG levels have been reported to exert, based on its similarities with benzodiazepines, anxiolytic, sedative[43,44] and anticonvulsant effects.[39,40] During postpartum an accelerated fall of PRG but also of ES, increases the risk of convulsion.

Another relevant finding was that the effects were more dependent on GABAA than on glutamate even during experiments where increment or defect of channel values were handled with the same intensity (20%) but the predominant effect was on receptors GABAA

The neuronal model implemented in the present study is restricted to PN cell responses. Additional effort is needed to generate not only a single multiple compartmental model to evaluate responses to ES and PRG for inhibitory interneuron (IN) but also a multicompartimental network responses to evaluate discharge patterns of different cell types i.e., PN and IN cells, with different expression of receptor types. However, these results support the notion that ES and/or PRG participate on amygdala principal neuronal responses involved in fear, anxiety and nocifens ive behavior. The proposed computational modeling study gives insight about possible effects of gender on amygdala neurons, perhaps a simplified way to study the complexity of hormonal effects on human behavior.

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Conflicts of interest

Authors declare no conflicts of interest.

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