INSTITUTO POTOSINO DE INVESTIGACIÓN CIENTÍFICA Y TECNOLÓGICA, A.C.

POSGRADO EN CIENCIAS EN BIOLOGIA MOLECULAR

Purinergic modulation of hippocampal synaptic activity

Tesis que presenta

Jessica Gabriela Rodríguez Meléndez

Para obtener el grado de

Maestra en Ciencias en Biología Molecular

Codirectores de la Tesis:

Dr. Carlos Barajas López

Dr. Marco Atzori

San Luis Potosí, S.L.P., junio de 2017

IPICYT

Constancia de aprobación de la tesis

La tesis ***"Purinergic Modulation of hipocampal activity"*** presentada para obtener el Grado de Maestra en Ciencias en Biología Molecular fue elaborada por **Jessica Gabriela Rodríguez Meléndez** y aprobada el **trece de julio de dos mil diecisiete** por los suscritos, designados por el Colegio de Profesores de la División de Biología Molecular del Instituto Potosino de Investigación Científica y Tecnológica, A.C.

Dr. Carlos Barajas López
Codirector de la tesis

Dr. Marco Atzori
Codirector de la tesis

Dra. Irene Beatriz Castano Navarro
Miembro del Comité Tutoral

Créditos Institucionales

Esta tesis fue elaborada en el Laboratorio de Neurobiología de la División de Biología Molecular del Instituto Potosino de Investigación Científica y Tecnológica, A.C., bajo la codirección del Dr. Carlos Barajas López (Proyectos no. 81409, 219859 CONACYT) y el Dr. Marco Atzori.

Durante la realización del trabajo el autor recibió una beca académica del Consejo Nacional de Ciencia y Tecnología (No. de registro 590369) y del Instituto Potosino de Investigación Científica y Tecnológica, A. C.

IPICYT

Instituto Potosino de Investigación Científica y Tecnológica, A.C.

Acta de Examen de Grado

El Secretario Académico del Instituto Potosino de Investigación Científica y Tecnológica, A.C., certifica que en el Acta 165 del Libro Primero de Actas de Exámenes de Grado del Programa de Maestría en Ciencias en Biología Molecular está asentado lo siguiente:

En la ciudad de San Luis Potosí a los 13 días del mes de julio del año 2017, se reunió a las 10:00 horas en las instalaciones del Instituto Potosino de Investigación Científica y Tecnológica, A.C., el Jurado integrado por:

Dra. Irene Beatriz Castaño Navarro	**Presidenta**	**IPICYT**
Dr. Marco Atzori	**Secretario**	**UASLP**
Dr. Carlos Barajas López	**Sinodal**	**IPICYT**

a fin de efectuar el examen, que para obtener el Grado de:

MAESTRA EN CIENCIAS EN BIOLOGÍA MOLECULAR

sustentó la C.

Jessica Gabriela Rodríguez Meléndez

sobre la Tesis intitulada:

Purinergic Modulation of hipocampal activity

que se desarrolló bajo la dirección de

Dr. Carlos Barajas López
Dr. Marco Atzori (UASLP)

El Jurado, después de deliberar, determinó

APROBARLA

Dándose por terminado el acto a las 11:15 horas, procediendo a la firma del Acta los integrantes del Jurado. Dando fe el Secretario Académico del Instituto.

A petición de la interesada y para los fines que a la misma convengan, se extiende el presente documento en la ciudad de San Luis Potosí, S.L.P., México, a los 13 días del mes de julio de 2017.

Dr. Horacio Flores Zúñiga
Secretario Académico

Mtra. Ivonne Lizette Cuevas Vélez
Jefa del Departamento del Posgrado

INSTITUTO POTOSINO DE INVESTIGACIÓN CIENTÍFICA Y TECNOLÓGICA, A.C.
IPICYT
SECRETARIA ACADEMICA

Dedicatorias

A mis padres Francisca Meléndez Vélez y Gabriel Rodríguez Flores.

A mis hermanos, Tania, Susana, Ana y Juan.

A mi tía Agapita y a mi prima Eliana.

A Omar Ramos.

Agradecimientos

Al Doctor Carlos Barajas López por permitirme trabajar bajo su dirección, por su paciencia y enseñanzas.

Al Doctor Marco Atzori por sus recomendaciones y la oportunidad de trabajar bajo su dirección.

A la Doctora Irene Castaño Navarro por su asesoría y sugerencias para mejorar este trabajo.

A la Doctora Marcela Miranda Morales por su paciencia al enseñarme, su disposición para resolver mis dudas y por su atención en este proyecto.

A Rosa Espinosa Luna por su ayuda y apoyo durante la maestría, sus consejos y por su amistad.

A CONACYT y al IPICYT por aceptarme como estudiante de maestría y la beca otorgada.

A mi familia por su apoyo todo este tiempo, a mis compañeros del laboratorio de Neurobiología y del IPICYT por brindarme su amistad y su apoyo.

A Omar por su apoyo constante.

Contenido

Constancia de aprobación de la tesis ii

Créditos Institucionales iii

Dedicatorias v

Agradecimientos vi

Lista de figuras viii

Resumen ix

Abstract x

Introduction 1

Materials and Methods 4

Slice preparation. 4

Whole-cell recordings and drug application. 4

Data analysis and statistics. 5

Results 6

Excitatory and spontaneous postsynaptic currents in CA1 hippocampal neurons 6

ATP (100 μM) significantly reduces the amplitude and frequency of glutamate-mediated spontaneous responses. 6

ATP (100 μM) reduced, in a reversible manner, the amplitude of glutamate-mediated evoked currents. 7

Discussion 8

Bibliography 18

Lista de figuras

Figure 1.Schematic representation of the main excitatory connections of a hippocampal coronal section. 10
Figure 2. Histogram frequency of postsynaptic spontaneous currents amplitudes recorded in CA1 hippocampal neurons. 11
Figure 3. Excitatory postsynaptic currents recorded in a CA1 neuron from the hippocampal *stratum pyramidal*. **¡Error! Marcador no definido.**
Figure 4. ATP (100 µM) significantly reduces the amplitude of glutamate-mediated spontaneous postsynaptic currents in CA1 neurons. 13
Figure 5.ATP (100 µM) significantly reduces the frequency of glutamate-mediated spontaneous postsynaptic currents in CA1 cells. 14
Figure 6. ATP transiently reduces the glutamate-mediated excitatory postsynaptic currents. 15
Figure 7. ATP inhibits with different kinetics the induced and spontaneous synaptic activity. 16
Figure 8. Comparison between induced and spontaneous responses 31 minutes after ATP removal. 17

Resumen

Modulación purinérgica de la actividad sináptica hipocampal

El ATP actúa como un neurotransmisor rápido y neuromodulador en el hipocampo, un área cerebral implicada en el aprendizaje y la memoria. Sin embargo, los receptores purinérgicos y la transducción de señales implicados en esta neuromodulación no son bien conocidos. En el presente trabajo, se estudiaron los efectos moduladores de la ATP sobre la actividad sináptica hipocampal espontanea e inducida. Se obtuvieron registros de Patch-clamp en su modalidad de célula completa de neuronas hipocampales CA1 en rebanadas de hipocampo de ratones C57BL / 6J (8 semanas de edad, macho o hembra). El ATP inhibió la amplitud y la frecuencia de las corrientes postsinápticas excitatorias espontáneas (EPSCs).Tal efecto no fue reversible. El ATP también inhibió la amplitud de EPSC evocadas, pero tal efecto parece ser reversible. En conclusión, la ATP inhibe la transmisión sináptica glutamatérgica en las neuronas del hipocampo CA1. Se requieren experimentos adicionales para determinar por qué los efectos sobre las EPSC espontáneas y evocadas parecen lavarse con diferentes cinéticas.

Palabras clave: ATP, EPSCs, CA1, NMDA, AMPA, P2Y.

Abstract

Purinergic Modulation of hippocampal activity

ATP acts as a fast neurotransmitter and neuromodulator in the hippocampus, a brain area implicated in learning and memory. However, the purinergic receptors and their signal transduction involved in this neuromodulation are not well understood. In the present work, we studied the modulatory effects of ATP on spontaneous and induced hippocampal synaptic activity. Whole-cell patch clamp recordings were obtained of CA1 hippocampal neurons in hippocampal slices of C57BL/6J mice (8 weeks old, male or female). ATP inhibited the amplitude and frequency of spontaneous excitatory postsynaptic currents (EPSCs). Such an effect was not reversible. ATP also inhibited the amplitude of evoked EPSCs but such an effect appears to be reversible. In conclusion, ATP inhibits the glutamatergic synaptic transmission in CA1 hippocampal neurons. Additional experiments are required to determine why the effects on spontaneous and evoked EPSCs appear to wash with different kinetics.

Key words: ATP, EPSCs, CA1, NMDA, AMPA, P2Y.

Introduction

Synapses are responsible for transmitting information quickly and efficiently among neurons (Pang and Han, 2012). In the brain, rapid inhibitory neurotransmission is mainly mediated by ionotropic receptors activated by γ-aminobutyric acid ($GABA_A$), which permit the entry of Cl^- ions and causes hyperpolarization of postsynaptic neurons (Pang and Han, 2012). Brain excitatory neurotransmission is mainly mediated by ionotropic glutamate receptors such as α-amino-3-hydroxyl-5-methylisoxazole-4-propionic acid receptors (AMPARs), kainic acid receptors (KARs) and N-methyl- Aspartate receptors (NMDARs). AMPARs are composed of four subunits, GluR1-GluR4 (glutamate receptor 1-4) and mediate most of the excitatory synaptic potentials in the brain (Wisden and Seeburg, 1993). Activation of postsynaptic AMPARs and NMDARs mediates the non-selective entry of cations resulting in excitatory postsynaptic currents leading to postsynaptic depolarization.

ATP is a neurotransmitter in CNS and co-released with other neurotransmitters (Burnstock, 2009). For instance, in the hippocampus, a brain area related to learning and memory, ATP is co-released with glutamate (Mori et al., 2001) and mediates fast synaptic transmission (Pankratov et al. 1998; Mori et al. 2001). ATP is released from hippocampal neurons (Khakh et al., 2003) and astrocytes (Koizumi et al. 2003; Zhang et al. 2003) through connexin/pannexin hemichannels (Montero and Orellana, 2015) or exocytosis. Endogenous ATP activates purinergic receptors, such as P2X receptors, which are ionotropic channels with high permeability for Ca2+. These channels are formed by three subunits of the same subtype (homomeric receptors) or by different subunits

(heteromeric). In mammals, there are seven P2X subunits described (P2X1-P2X7). Each subunit has an intracellular COOH and NH3 termini bound by two transmembrane segments (TM1 and TM2) separated by an ectodomain (~ 280 amino acids) where ATP is bound (North et al., 2002).

Another family of purinergic receptors is the metabotropic P2Y receptors, which are G-protein-coupled and have 7 transmembrane domains. So far, eight subtypes of P2Y receptors (P2Y1, 2, 4, 6, 11, 12–14) have been described (Burnstock et al., 2011). After the release of ATP, this is rapidly hydrolyzed to adenosine by ectonucleotidases in the extracelullar space (Dunwiddie et al., n.d.). Adenosine activates P1 (A1.A2A, A2B, A3 subtypes), which are also G-protein coupled receptors with 7 transmembrane domains with short N termini (7-13 residues) and C-termini of 32-120 residues (Burnstock, 2007). In the hippocampus all types of P2X receptors are expressed, also P2Y1, P2Y2, P2Y4, P2Y6 and P2Y12 receptors (Burnstock et al., 2011) and A1 receptor are highly expressed in this area (Svenningsson et al., 1997).

Another role of the ATP, and probably the most important in the hippocampus, is as a neuromodulator, through P2X receptors, which mediate long term potentiation (LTP) or long term depression (LTD) (Pankratov et al. 2002; Yamazaki & Fujii 2003; Fujii 2004) and release of other neurotransmitters at the presynaptic level (Guzman and Gerevich, 2016) or at postsynaptic level interacting with neurotransmitter receptors (Pankratov et al. 2009; Pougnet et al. 2016). Furthermore, ATP also can modulate synaptic transmission by inhibition of

neurotransmitter release through P2Y receptors (Fernandez-Mendoza et al., 2000) or by inhibition of other neurotransmitter receptors (Mundell et al. 2004; Luthardt et al. 2003). However, the role of ATP in hippocampus as a neuromodulator is not well understood and purinergic receptors involved and mechanisms of action remain unknown. In this study, we investigated the modulatory effect of ATP on spontaneous excitatory postsynaptic currents (EPSCs) and on the evoked EPSCs in CA1 hippocampal neurons.

Materials and Methods

Slice preparation. Slices were obtained from adult (8 weeks old, male or female) C57BL/6J mice. After of cervical dislocation, the entire brain was quickly removed and placed in ice-cold (0-4°C) oxygenated (95 % O2–5% CO2) artificial cerebrospinal fluid (ACSF) containing (mM): 126 NaCl, 3.5 KCl, 1.25 NaH2PO4, 1.5 Mg2Cl2, 1.5 CaCl2, 25 NaHCO3 and 10 dextrose (pH ~7.4). Coronal hippocampal slices were prepared with a vibroslicer (vibroslice NVSLM1) and maintained in an incubation chamber with oxygenated ACSF at room temperature for at least 1 h before experiments. A single slice was transferred to recorder chamber with a slice holder and continuously perfused with oxygenated ACSF at a rate of 2.0–3.0 ml min-1 at room temperature. The volume of the recording chamber was about ~3 ml and maintained constant by a needle connected to a vacuum pump.

Whole-cell recordings and drug application. All experiments were recorder from CA1 neurons, with glass pipette (3-5 MΩ) filled with solution containing (mM): 100 CsCl, 5 BAPTA, 1 lidocaine QX-314, 1 $MgCl_2$, 10 Hepes, 4 L-Glutathione reduced, 3 ATPMg, 0.3 GTPNa and 20 Phosphocreatine. Excitatory postsynaptic currents (EPSCs) were evoked by electrical stimulation of the Shaffer collateral-commissural afferents, using tungsten bipolar electrode of diameter of 75 µm. Electrical pulses (100-200 µs) were delivered every 3 minutes and generated by an isolated stimulator (Isostim A320). Evoked and spontaneous currents were recorded using an Axopatch 200B amplifier (Axon Instruments, CA), digitalized

with an analog-digital converter (Digidata 1320), and stored in a PC using pCLAMP 8 software (Axon instruments). The strength of stimulation was adjusted to obtain about 40-60% of the maximum response. Evoked and spontaneous currents were recorded for 6 min and then picrotoxin (30 µM) was superfused for 15 minutes before applying ATP (100 µM) for 10 minutes. ATP was washed out during 20 minutes.

Data analysis and statistics. Results were expressed as mean ± S.E.M and the numbers of cells are indicated as *n* number. Statistical comparisons were done using one-way ANOVA with post hoc Dunnett test, or two-way ANOVA with post hoc Bonferroni test, as appropriate. Spontaneous currents were analyzed using MiniAnalysis software, and evoked currents were analyzed using Clampfit (pCLAMP 10 software). Graphs and statistics were prepared using GraphPad Prism 6 software and printings of evoked and spontaneous currents were obtained using CANVAS.

Results

Excitatory and spontaneous postsynaptic currents in CA1 hippocampal neurons

A total of 14 CA1 hippocampal neurons from *stratum pyramidale* (**Fig 1**) were recorded for at least 45 minutes, all of which showed spontaneous synaptic activity. Spontaneous postsynaptic currents were recorded in seven neurons, which had an average amplitude of 51 ± 12.4 pA (range from 9-100 pA), but the most frequent are 20-39 pA (**Fig 2**). In control conditions, their average duration was 30 ± 4.5 ms and a frequency of 1 ± 0.06 Hz. Excitatory postsynaptic currents (EPSCs) were measured in the other seven neurons and had an average amplitude of 252 ± 61 pA and a duration of 51 ± 7.1 ms. The amplitude of EPSCs was increased as we increased the intensity of the stimulus (**Fig 3**) until a maximum response was reached. Intensity was adjusted to evoke about 40-60 % of the maximum response to avoid over stimulation and to evaluate ATP effects.

ATP (100 μM) significantly reduces the amplitude and frequency of glutamate-mediated spontaneous responses.

ATP (100 μM) effects on glutamate-mediated spontaneous currents were investigated by first blocking GABA receptors with picrotoxin (30 μM). ATP reduced the amplitude as well as the frequency of spontaneous currents. The mean amplitude (**Fig 4**) and frequency (**Fig 5**) of spontaneous postsynaptic currents

exhibited a significant decrease ($P < 0.05$, $n=7$) 5 to 8 minutes after ATP application, inhibition that was not reversible during 20 min of wash out.

ATP (100 µM) reduced, in a reversible manner, the amplitude of glutamate-mediated evoked currents.

To evaluate the modulatory effect of ATP on glutamate-mediated induced response, we applied ATP (100 µM) during 10 minutes on evoked excitatory postsynaptic currents recorded in CA1 cells in presence of picrotoxin, ATP induced a significant ($P < 0.05$) decrease of induced responses (**Fig 6**) . This inhibition was maximal 10 minutes after ATP application, current amplitude returned almost to basal values 25 minutes after beginning ATP removal from the recording chamber.

When we plotted the normalized spontaneous and evoked currents during ATP wash out (**Fig 7**), it was evident that ATP induced a larger decrease of the evoked than spontaneous currents ($P < 0.05$) and the evoked currents tended to recover faster than spontaneous currents (**Fig 8**). Indeed, 31 minutes after ATP removal, evoked currents reached about 90% of the control amplitude, however, we need to increase the number of experiments to reach statistical significance ($P = 0.16$).

Discussion

The present findings provide evidence that ATP is able to modulate both spontaneous and induced synaptic glutamatergic transmission in CA1 hippocampal neurons. The amplitude and frequency of spontaneous EPSCs were reduced and this effect was not reverted even after 25 min of ATP wash out, we propose that this effect resembles the long term depression (LTD). However, ATP causes a larger but transitory inhibition of evoked EPSCs (irreversible decrease) an induced ESPCs indicating that these two effects might be mediated by different mechanisms.

These effects could be mediated by metabotropic (P2Y or adenosine) receptors as infusion of ATP through the bath would be expected to desensitize P2X (ionotropic) receptors. ATP is able to modulate neurotransmitter release at the presynaptic level (Fernandez-Mendoza et al., 2000), at the postsynaptic level it modulates fast neurotransmitter receptors (Guzman and Gerevich, 2016), thus the effects of ATP on spontaneous and induced activity could be at the pre or postsynaptic level. In support to a postsynaptic action, expression of P2Y1 receptor has been found in pyramidal cells of hippocampus (Moore et al. 2000; Matute 2000). Nevertheless, in this area, ATP is known to inhibit glutamate by acting through P2Y receptors located in nerve terminals arriving from CA3 to CA1 neurons (Fernandez-Mendoza et al., 2000). Reduction of amplitude of induced responses probably is due to this inhibition of glutamate release, but a postsynaptic effect is not ruled out because P2Y receptors are able to modulate synaptic transmission by inhibition of postsynaptic NMDA and AMPA receptors in CNS

(Guzman and Gerevich, 2016). The fact that ATP decreases drastically the frequency of spontaneous responses, suggests that its effect is predominantly presynaptic. This presynaptic effect could also be mediated by adenosine receptors (A1), as in CA3 hippocampal neurons (Kawamura et al., 2004) or through a excitation of interneurons by P2Y1 receptors that increase inhibitory synaptic transmission in CA1 neurons (Bowser and Khakh, 2004). However, this mechanism is not likely to be the explanation for the effects observed here because we applied ATP in the presence of picrotoxin, which would block GABAergic neurotransmission (Rombo et al., 2016). This study is a preliminary study of the ATP effects on both spontaneous and induced EPSCs in hippocampal neurons.

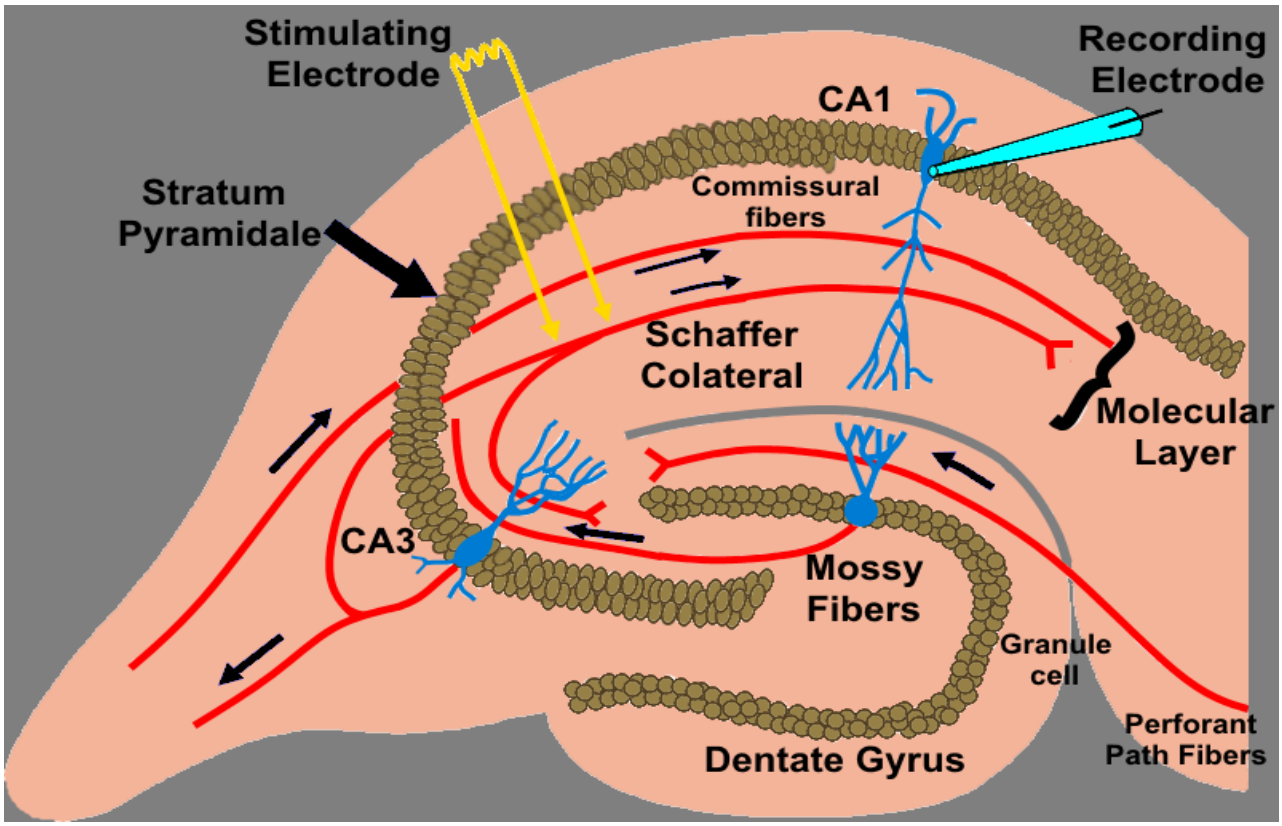

Figure 1.Schematic representation of the main excitatory connections of a hippocampal coronal section.

Two important layers are present in the hippocampus, stratum pyramidale and the molecular layer. The stratum pyramidale is a continuous neuronal layer that contains CA1 and CA3 pyramidal neurons, which is the main cell type. The pyramidal neuron has two dendritic trees that emerge from opposite poles of the cell body. Perforant path fibers synapse on granule cell of the dentate gyrus; whereas axons of granule cells form mossy fibers and send their inputs to CA3 pyramidal neurons, which form synapses with themselves and with CA1 pyramidal neurons through branches called Schaffer collaterals (Luo, 2016). A single CA1 pyramidal neuron is innervated by about 5000 different CA3 neurons. For this study, whole-cell recordings were made from CA1 pyramidal neurons. Stimulating bipolar electrode was positioned in Shaffer collaterals (Hammond, 2001).

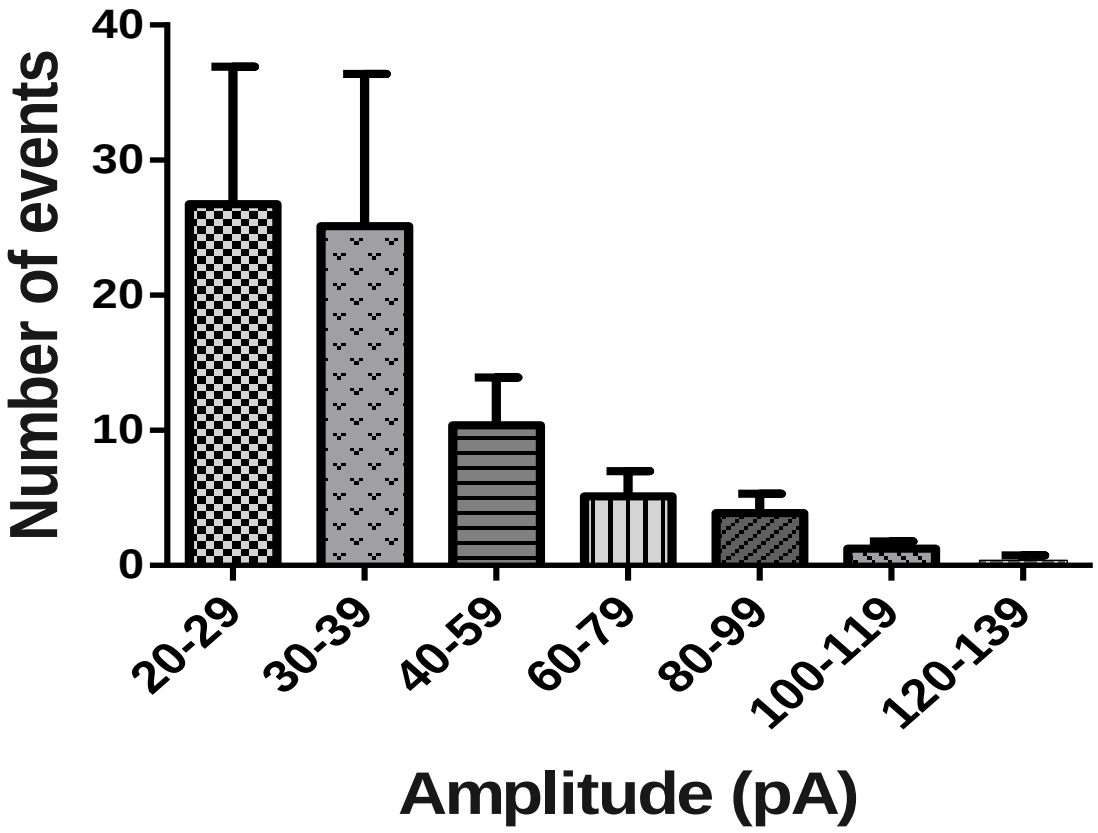

Figure 2. Histogram frequency of postsynaptic spontaneous currents amplitudes recorded in CA1 hippocampal neurons.

Currents larger than 20 pA were considered. Bars and top lines represent the mean and S.E.M (n=7). Currents were analyzed in 2 min periods.

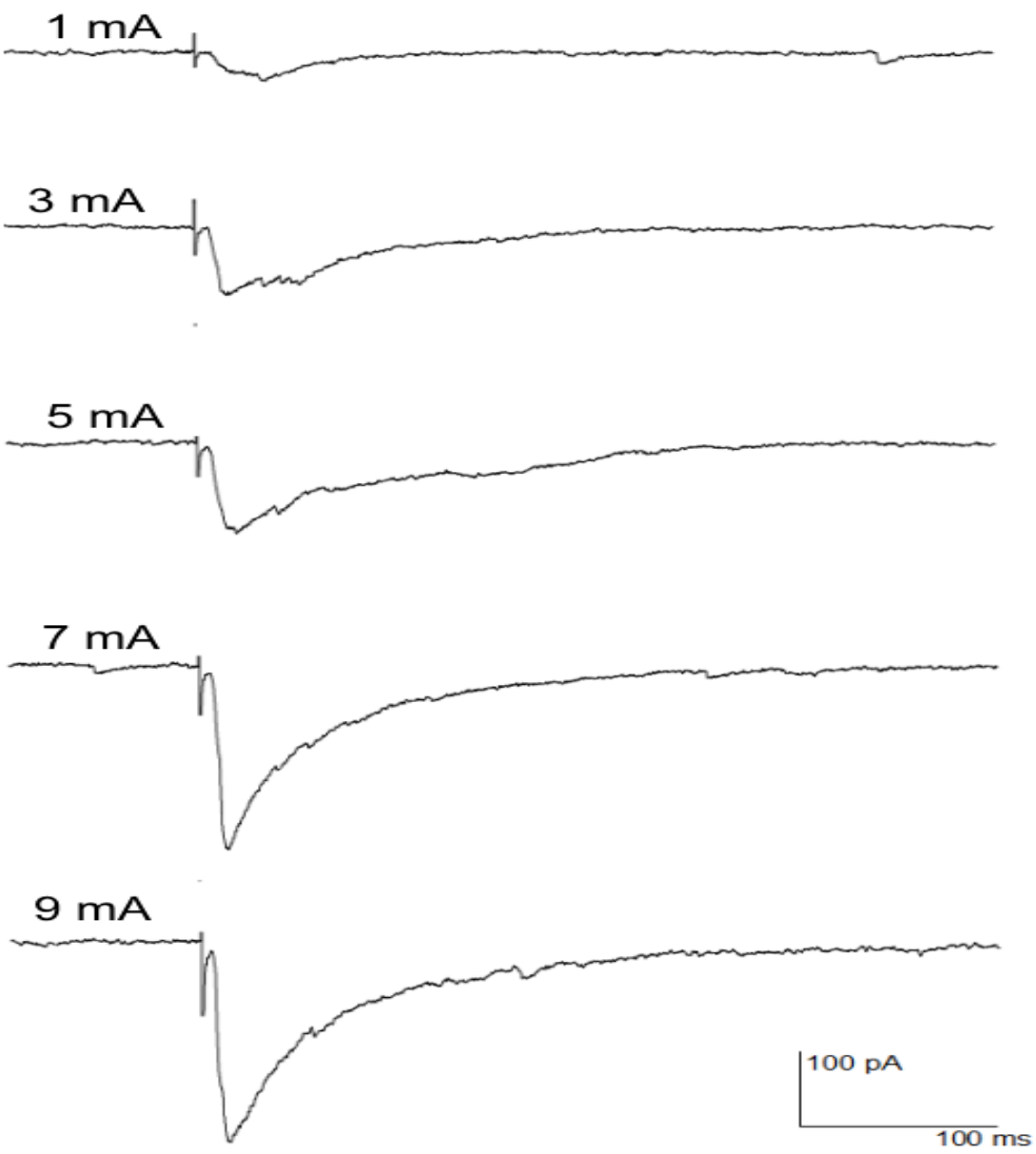

Figure 3. Excitatory postsynaptic currents recorded in a CA1 neuron from the hippocampal *stratum pyramidal*.

Currents were evoked by single electrical pulses of 200 µs and the indicated intensities (mA). Synaptic currents are larger when pulse amplitude is increased. For this study, we used a stimulus intensity that generated about 40-60% of the maximal response (5 mA for this sample).

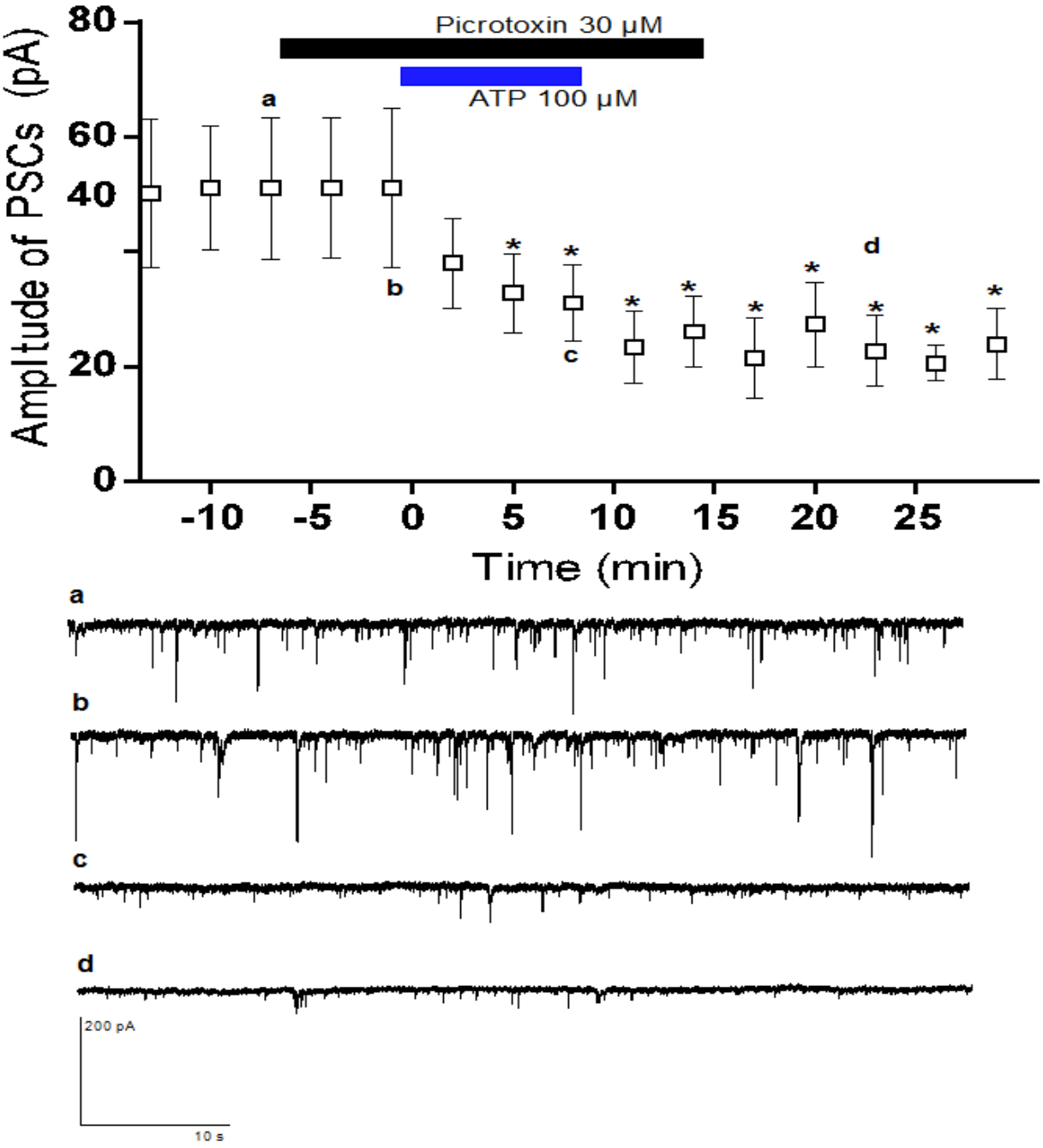

Figure 4. ATP (100 µM) significantly reduces the amplitude of glutamate-mediated spontaneous postsynaptic currents in CA1 neurons.

The plot shows the average amplitude (pA) as a function of time. ATP and picrotoxin were applied as indicated by bars. Squares and lines represent the mean and S.E.M. (*=P<0.05; n=7). Lower traces are representative recordings of spontaneous currents from one CA1 neuron, at the time indicated by letters.

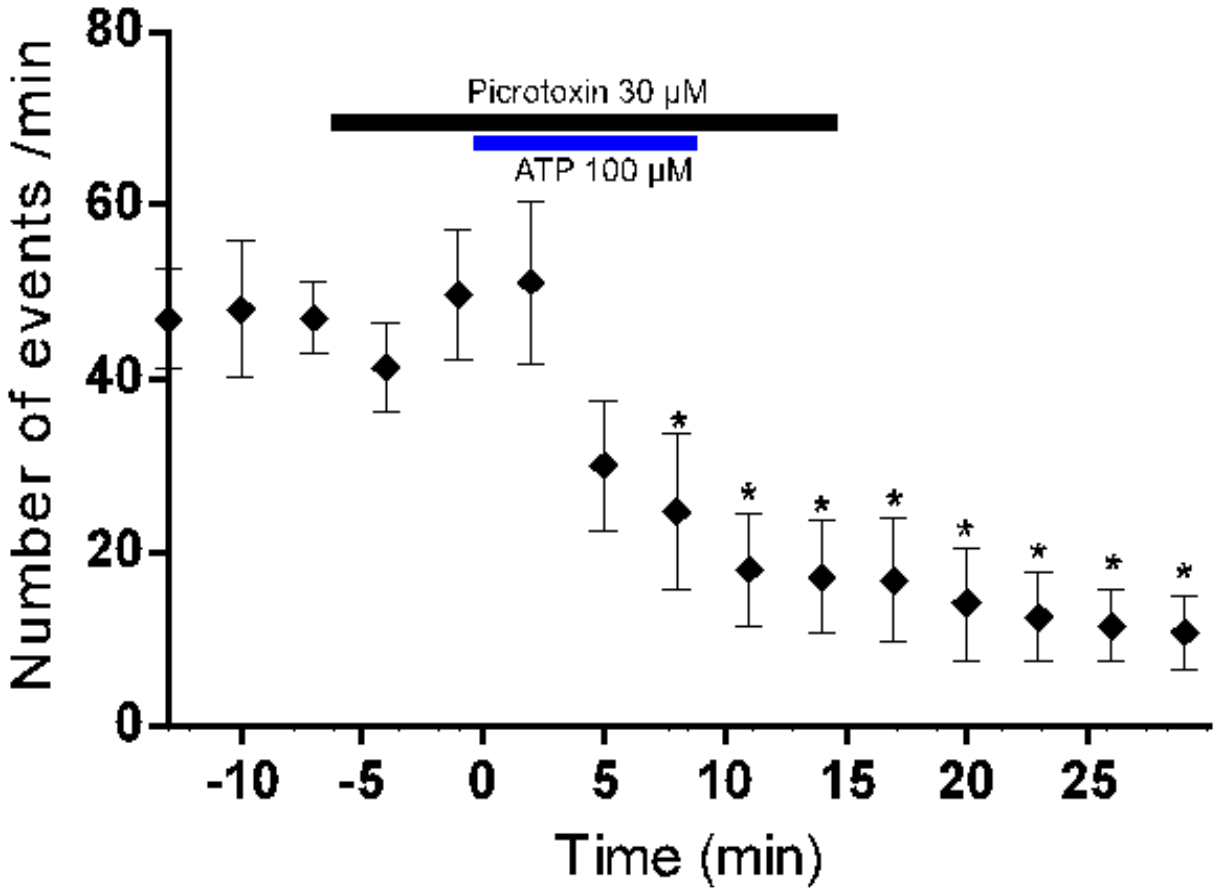

Figure 5.ATP (100 µM) significantly reduces the frequency of glutamate-mediated spontaneous postsynaptic currents in CA1 cells.

Number of events is show as a function of time. ATP and Picrotoxin were applied as indicated by the bars. Squares and bars represent the mean and S.E.M. ($p<0.05$; $n=7$).

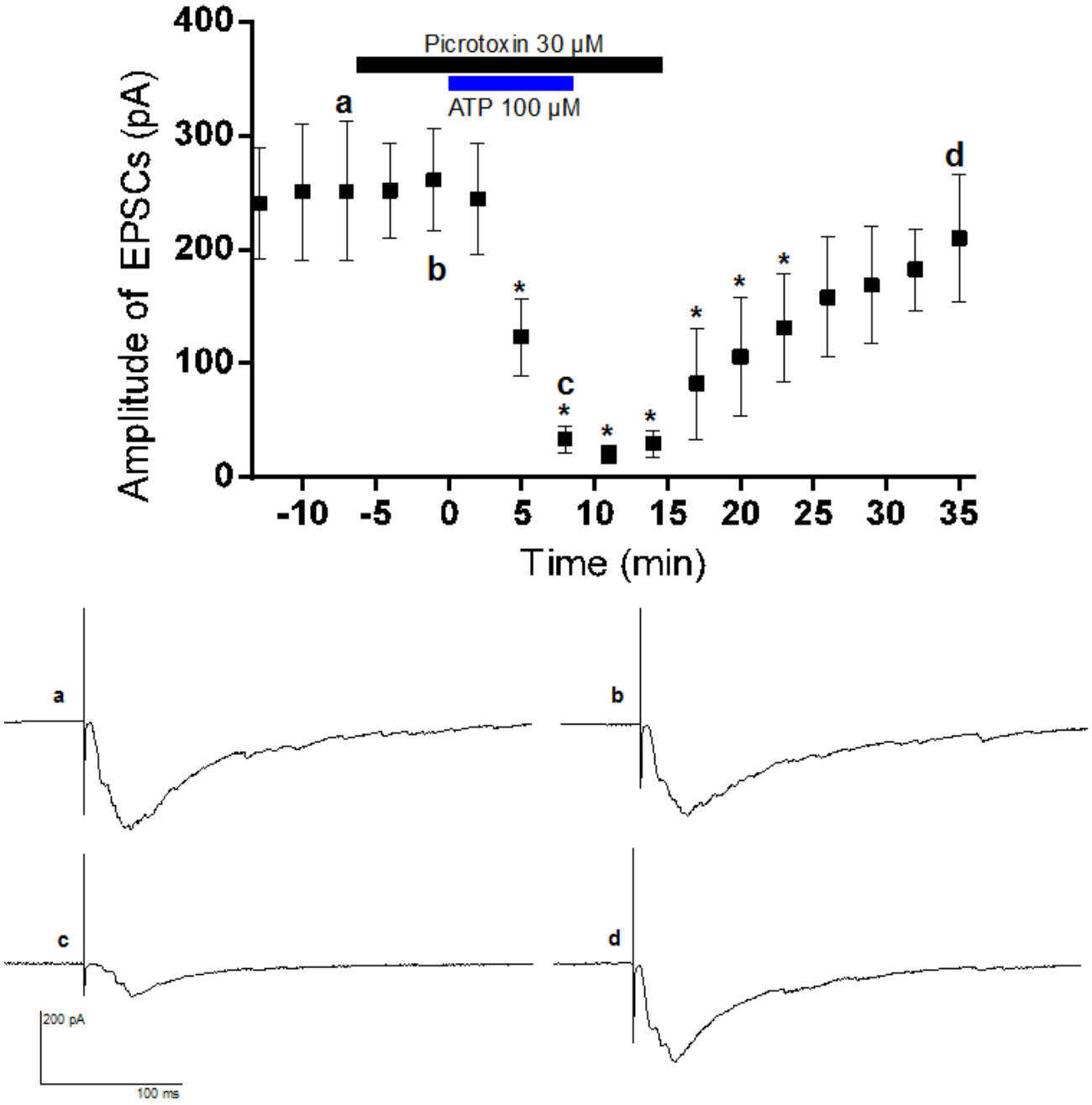

Figure 6. ATP transiently reduces the glutamate-mediated excitatory postsynaptic currents.

The plot shows the amplitude (pA) as a function of time. ATP and Picrotoxin were applied as indicated by the bars. Squares and bars represent the mean and S.E.M. ($p<0.05$; $n=7$). Lower traces are representative EPSCs from one CA1 cell at the times indicated by the letters.

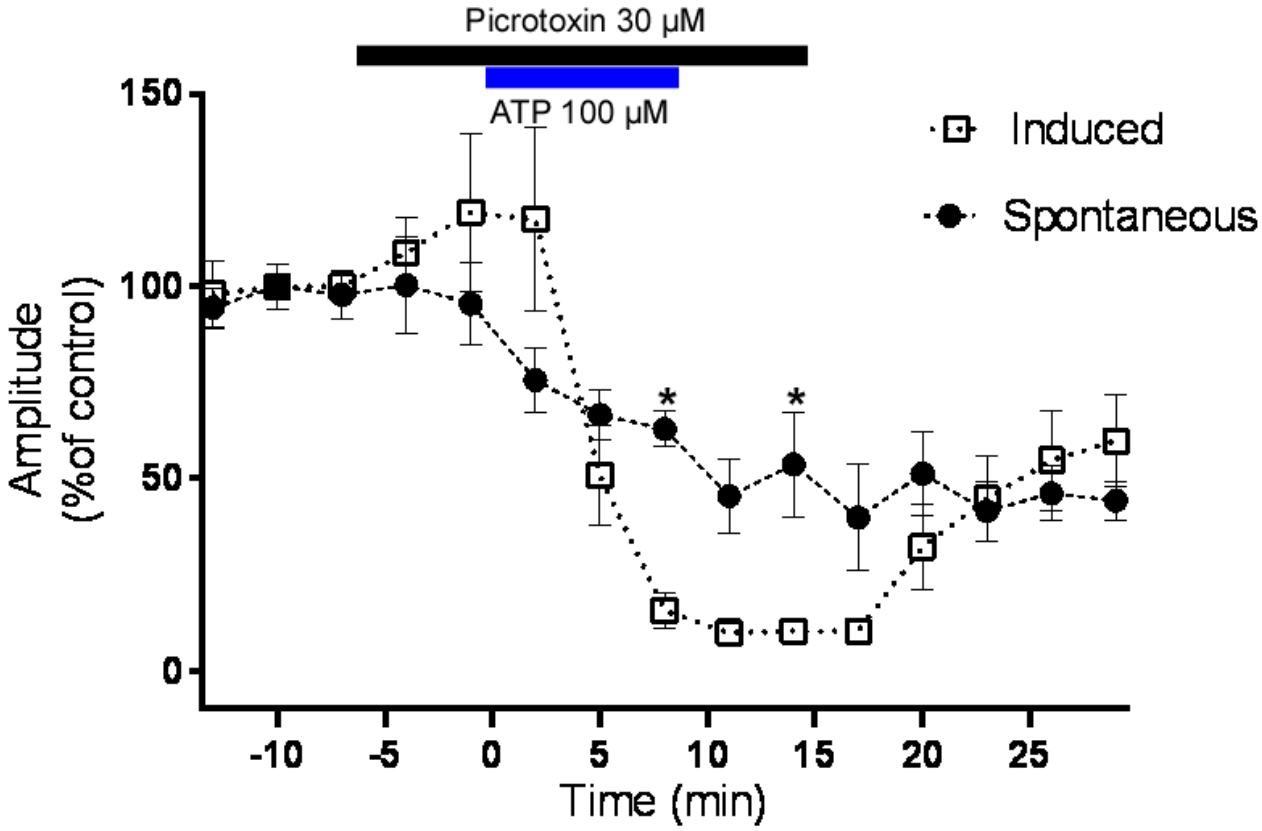

Figure 7. ATP inhibits with different kinetics the induced and spontaneous synaptic activity.

The plot shows the average amplitude of the induced and spontaneous currents (% of control values) before, during, and after ATP perfusion. ATP and picrotoxin were applied as indicated by the bars. Squares and bars represent the mean and S.E.M. ($p<0.05$; $n=7$). The spontaneous response is gradually reduced and does not recover during the ATP wash out, whereas the induced synaptic currents are rapidly reduced and partially recover.

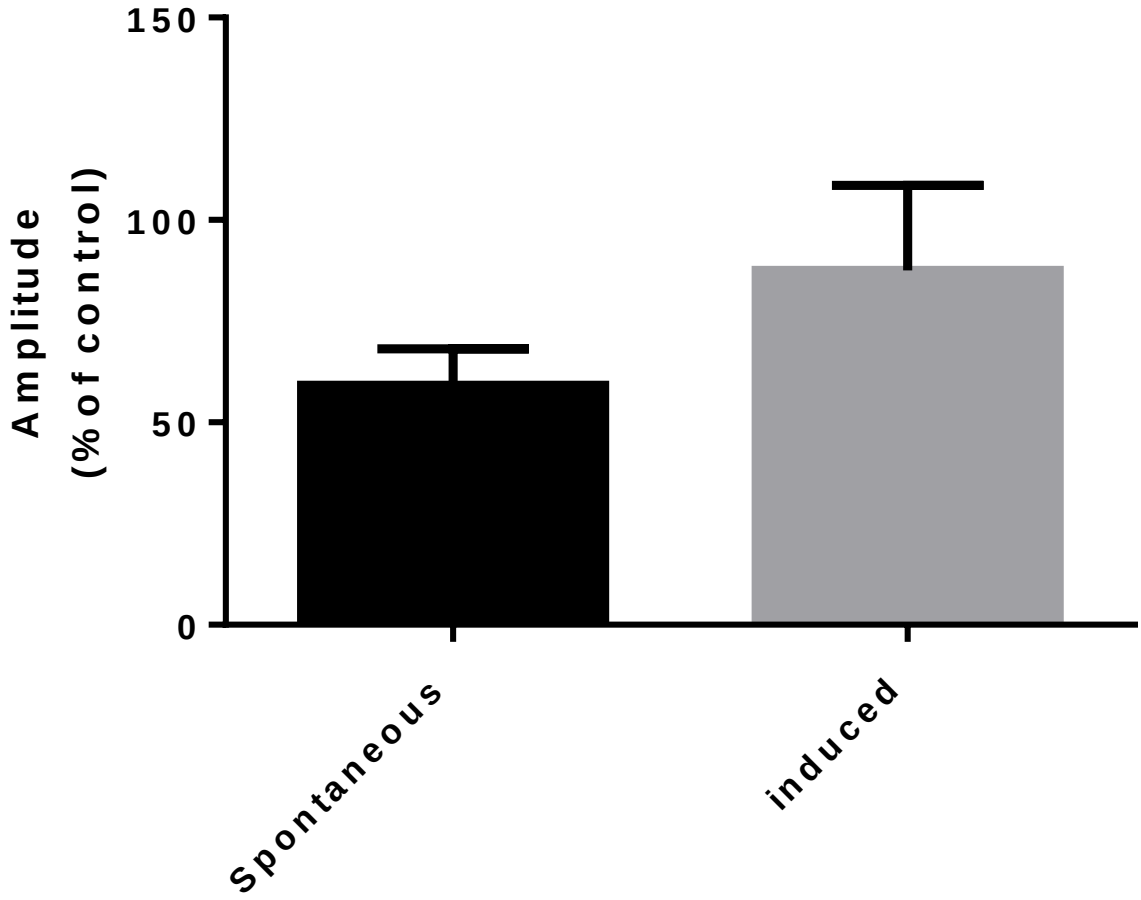

Figure 8. Comparison between induced and spontaneous responses 31 minutes after ATP removal.

Columns and top lines represent the mean and S.E.M. Spontaneous (n=3) and induced (n=4) amplitude (% of control) were compared (P = 0.16). Induced responses tend to recover until basal level; instead the inhibition of the spontaneous currents was maintained over time, we need to increase the number of experiments to confirm these differences.

Bibliography

Bowser DN, Khakh BS (2004) ATP Excites Interneurons and Astrocytes to Increase Synaptic Inhibition in Neuronal Networks. 24:8606–8620.

Burnstock G (2007) Purine and pyrimidine receptors. Cellular and Molecular Life Sciences 64:1471–1483.

Burnstock G (2009) Purinergic cotransmission. 4:2–5.

Burnstock G, Fredholm BB, Verkhratsky A (2011) Adenosine and ATP Receptors in the Brain. :973–1011.

Dunwiddie T V, Diao L, Proctor WR (n.d.) Adenine Nucleotides Undergo Rapid, Quantitative Conversion to Adenosine in the Extracellular Space in Rat Hippocampus.

Fernandez-Mendoza VM, Andrew RD, Barajas-López C (2000) ATP Inhibits Glutamate Synaptic Release by Acting at P2Y Receptors in Pyramidal Neurons of Hippocampal Slices. 293:172–179.

Fujii S (2004) Forum Mini review ATP- and Adenosine-Mediated Signaling in the Central Nervous System : The Role of Extracellular ATP in Hippocampal Long-Term Potentiation. 106:103–106.

Guzman SJ, Gerevich Z (2016) P2Y Receptors in Synaptic Transmission and Plasticity : Therapeutic Potential in Cognitive Dysfunction. 2016.

Hammond C (2001) Cellular and molecular neurobiology. Academic Press.

Kawamura M, Gachet C, Inoue K, Kato F (2004) Direct Excitation of Inhibitory Interneurons by Extracellular ATP Mediated by P2Y 1 Receptors in the Hippocampal Slice. 24:10835–10845.

Khakh BS, Gittermann D, Cockayne DA, Jones A (2003) ATP

Modulation of Excitatory Synapses onto Interneurons. 23:7426–7437.

Koizumi S, Fujishita K, Tsuda M, Shigemoto-mogami Y, Inoue K (2003) Dynamic inhibition of excitatory synaptic transmission by astrocyte-derived ATP in hippocampal cultures. :3–8.

Luo L (2016) Memory , Learning , and Synaptic Plasticity.

Luthardt J, Borvendeg SJ, Sperlagh B, Poelchen W, Wirkner K, Illes P (2003) P2Y 1 receptor activation inhibits NMDA receptor-channels in layer V pyramidal neurons of the rat prefrontal and parietal cortex. Neurochemistry International 42:161–172.

Matute C (2000) Immunohistochemical localization of the P2Y 1 purinergic receptor in neurons and glial cells of the central nervous system. 78:50–58.

Montero TD, Orellana JA (2015) Hemichannels: New pathways for gliotransmitter release. Neuroscience 286:45–59.

Moore D, Chambers JON, Waldvogel H, Faull R (2000) Regional and Cellular Distribution of the P2Y 1 Purinergic Receptor in the Human Brain : Striking Neuronal Localisation. 384:374–384.

Mori M, Heuss C, Gähwiler BH, Gerber U (2001) Fast synaptic transmission mediated by P2X receptors in CA3 pyramidal cells of rat hippocampal slice cultures. :115–123.

Mundell SJ, Pula G, Mcilhinney RAJ, Roberts PJ, Kelly E (2004) Desensitization and Internalization of Metabotropic Glutamate Receptor 1a Following Activation of Heterologous G q / 11 - Coupled Receptors †. :7541–7551.

North RA et al. (2002) Molecular physiology of P2X receptors. Physiological reviews 82:1013–1067.

Pang ZP, Han W (2012) Regulation of synaptic functions in central nervous system by endocrine hormones and the maintenance of energy homoeostasis. Bioscience reports 32:423–432.

Pankratov Y, Castro E, Miras-portugal MT, Krishtal O (1998) SHORT COMMUNICATION A purinergic component of the excitatory

postsynaptic current mediated by P2X receptors in the CA1 neurons of the rat hippocampus. 10:3898–3902.

Pankratov Y, Lalo U, Krishtal OA (2009) P2X RECEPTORS AND SYNAPTIC PLASTICITY. 158:137–148.

Pankratov Y V, Lalo U V, Krishtal OA (2002) Role for P2X Receptors in Long-Term Potentiation. 22:8363–8369.

Pougnet J, Compans B, Martinez A, Choquet D, Hosy E, Boué-grabot E (2016) P2X-mediated AMPA receptor internalization and synaptic depression is controlled by two CaMKII phosphorylation sites on GluA1 in hippocampal neurons. Nature Publishing Group:1–13.

Rombo DM, Ribeiro JA, Medicina F De, Lisboa U De, Medicina F De, Lisboa U De, Medicina F De, Lisboa U De (2016) Hippocampal GABAergic transmission: a new target for adenosine control of excitability.

Svenningsson P, Hall H, Sedvall G, Fredholm BB (1997) Distribution of adenosine receptors in the postmortem human brain: An extended autoradiographic study. Synapse 27:322–335.

Wisden W, Seeburg PH (1993) Mammalian ionotropic glutamate receptors. Current opinion in neurobiology 3:291–298.

Yamazaki Y, Fujii S (2003) Long-Term Potentiation and Long-Term Depression Induced by Local Application of ATP to Hippocampal CA1 Neurons of the Guinea Pig. 92:81–92.

Zhang J-M, Wang H-K, Ye C-Q, Ge W, Chen Y, Jiang Z-L, Wu C-P, Poo M-M, Duan S (2003) ATP Released by Astrocytes Mediates Glutamatergic Activity-Dependent Heterosynaptic Suppression. Neuron 40:971–982.