Lab Report

Electrophysiological characterization of a GluA4 gamma 2 tandem and investigation of first event amplitudes in single channel recordings

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| Scientific Topic (3-5 keywords):  transmembrane auxiliary AMPA proteins, AMPA subconductance levels, single channel recordings, xxx |
| Methods (3-5 keywords):  Macroscopic patch clamp recordings, single channel patch clamp recordings, xxxxx |

Please tick 2 fields at max.:

|  |  |
| --- | --- |
| ⌧ Cellular Neuroscience | 🞎 Clinical/Translational Neuroscience |
| ⌧ Molecular Neuroscience | 🞎 Cognitive Neuroscience |
| 🞎 Systems Neuroscience | 🞎 Computational Neuroscience |

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| Lab Leader:  Prof. Dr. Andrew Plested | Suggested Second Reviewer: |
|  |  |

**1 Introduction**

* 1. **AMPA significance, function and structure**

The α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) is an ionotropic glutamate receptor

Most widespread receptor in brian,

involved in so many different processes - learning

Structure – dimer of dimers – 3 domains (describe in detail structure and function of each) – ligand binding domain clamshell that pulls open the pore – sublevel activation (rosenmund)

* 1. **Slow AMPA**

Ampa is fast !

**1.3 Sebastians project**

Ampa mutant that has no desensitization

Zinc bridge – slower activation – 3 componentes of first latency distribution

We are only looking at the temporal structure of slowly activating condition – not the whole picture

**2 Materials and Methods**

**2.1 Cell Culture**

The human embryonic kidney 293 (HEK293) cell line was used for all patch clamp experiments. All cells were cultured in Modified Eagle Medium (MEM, manufacturer) supplemented with 6% fetal bovine serum (FBS, Manufacturer) in T-25 flasks (manufacturer) and incubated at 367ºC and 5% CO2 in an xxxxx incubator (manufacturer).

Cells were split approximately twice a week, when the confluency reached ~90%. The cells were washed with 5 mL of Dulbecco’s phosphate buffered saline (DPBS, manufacturer) and incubated on 250 xxxxTrypsin/ xxxx EDTA (manufacturer) at 367ºC for 3 minutes. Afterwards the cells were suspended in 10 mL MEM + 6% FBS to stop trypsination, after which they were centrifuged at 100g for 5 minutes in xxxx centrifuge (manufacturer). Supernatant was removed and the cells were resuspended in 5 mL MEM + 6% FBS and split 1:7 - 1:11 (depending on confluency) into a new T-25 flask.

Two days before transfection, 30 – 200 µL of cell suspension (depending on confluency) were seeded onto a ø25 mm dish (manufacturer) containing 2 mL MEM + 6% FBS and ~4 ø10 mm coverslips coated with polylysine. These coverslips had been previously sonicated for 15 minutes in 70% ethanol and had been kept in 99% ethanol at room temperature. For coating, coverslips were flamed and put into ø35 mm dishes. Subsequently, ~200 µL of xxxxx polylysine solution (manufacturer) were added to the coverslip, incubated at room temperature for 3 minutes, and removed. After washing twice in 2 mL DPBS, the dishes with coated coverslips were stored at 4ºC for up to 4 weeks.

**2.2 Transfection**

6 µL of xxx polyethylenimine (PEI) solution in xxxx were added to 192 µL of OPTI-MEM (manufacturer) and incubated for 3 minutes at room temperature. 2 µL of xxx plasmid solution totalling xxx ng DNA were added and incubated for another 20 minutes at room temperature. The total mixture was then added dropwise to the cells and incubated for 5-12 hours at 367ºC. Afterwards, the transfection medium was removed, the cells were washed twice in 2 mL DPBS prior to adding 2 mL of fresh medium supplemented with 30 µL xxxxx NBQX disodium solution in water.

The following plasmids were used:

**2.3 Electrophysiological setup**

Recordings were done 16-48 hours after transfection. Data was collected on the same electrophysiology setup using an Axopatch 200B patch clamp amplifier and amplifier output was monitored on a Tektronix TBS1102B digital oscilloscope. Digitization was performed using a HEKA InstruTECH ITC-18 interface.

Patch pipettes were pulled using a Sutter P-1000 pipette puller from G150F-3 capillaries xxxxxx

Electrodes were made from Teflon coated silver wire tipped with a silver chloride pellet. The head stage containing the electrode holder was moved using a Scientifica PatchStar micromanipulator. Pipettes typically had resistances of 3-6 MΩ, and were mounted onto a G23 Instruments electrode holder after being filled with intracellular solution (Table XXX). To block the inwardxxxx rectification of GluA4 in the presence of polyamines

Solution exchange was achieved using a Physik Instrumente piezoelectric stack actuator, which received XXX V pulses from the acquisition software, transmitted by a Siskiyou controller. The actuator moved a perfusion tool, which was made in the lab as laid out in detail in Plested and Poulsen 2021 (SOURCE), exposing the stationary patch pipette to two different extracellular solutions (Table XX). Solution 2 contained xxxx glutamate for receptor activation and xxx sucrose to make it easier to distinguish under the microscope.

For patching, coverslips containing cells were removed from the incubator and placed into a fresh ø35mm dish containing Solution 1 mounted on an Olympus IX71 microscope in a TMC Faraday cage on a TMC vibration-free table. Cells were illuminated using a Lumencore Sola Light Engine lamp and eGFP fluorescence was examined using blue excitation light emitted by xxxxxx. Healthy looking, transfected cells were selected by inspection. After an outside-out patch had been excised, the solution flow through the perfusion tool was started. After the recordings, the bath solutions were replaced to keep extracellular glutamate solutions low. All data was acquired using the AxoGraph acquisition software (AxoGraph Scientific, John Clements) at 40kHz after passing through an 8-pole Bessel filter at 10 kHz. The speed of solution exchange and positioning of the patch pipette was tested by applying the solutions to an open tip patch after recording.

**2.4 Electrophysiological recordings**

**2.5 Data Analysis**

Macroscopic current recordings were selected, averaged and baseline corrected in AxoGraph. Subsequently, statistical calculations like rise time, peak current, steady state current, as well as fitting xxxxxx exponential to the desensitization and decay curves and event detection were all performed using IgorPro64 (xxxxx) and compiled in Microsoft Excel (xxxxx). The code for further analysis and data visualization was written in python (version xx, xxx), using the python matplotlib library (xxxx), and is publicly available on the GitHub repository xxxxxxx.

**2.6 Single channel analysis**

All analysis for the single channel project was written in python. For the majority of the final data analysis, the python program ASCAM (source) was used. Data and results were visualized using the matplotlib library (xxxxx).

**Results**

**3.1 GluA4 gamma2 tandem kinetics**

**3.1.1 500 ms glutamate pulse**

**3.1.2 2 ms glutamate pulse**

**3.1.3 recovery from desensitization**

**3.1.4 Spike trains**

**3.1.5 I-V curves**

**3.2 Single channel analysis**

Rich data set, describe what he already did, his data structure

Problem of defining first event

**3.2.1 idealized trace at first activation**

**3.2.2 First event before drop in idealized trace**

**3.2.3 Redoing all the idealizations, implementation in ascam**

Implementing exclusion time

Implementing a new way to find first events

**4 Discussion**

**4.1 we didn’t find any slow ampa**

**4.2 single channel analysis**

**Limitations**

Effect of the resolution on the idealization, in general the idealization is quite flawed probably

Noise of the setup, limited samples etc

**Future directions**

Look at the distribution of first latencies for each first open state -> how many (resolvable) shut states before going into the corresponding open state

**Conclusion**

**References**