NMDA Receptor Modulators in QPatch

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Introduction

N-methyl-D-aspartate receptors (NMDARs) are ionotropic glutamate receptors permeable to Ca^{2+}, Na^{+} and K^{+}. To be activated, they need to bind to glutamate (via GluN2 subunits), glycine (via GluN1) and release the Mg^{2+} blockade by membrane depolarization. The majority of NMDARs are tetrameric complexes, consisting of two glycine-binding GluN1 subunits and two glutamate-binding GluN2 subunits. GluN1 is coded by a single gene with at least eight different splice variants; four different GluN2 genes originate GluN2A, GluN2B, GluN2C, and GluN2D subunits. NMDARs containing different GluN2 subunits have different pharmacological and kinetic properties.

NMDA receptor modulators can be studied using a variety of techniques. Many of them are available in Evotec, including those listed here below:

Platform	Readout	Throughput (samples/run)	Application(s)
FLIPR	Calcium	384	L. / N.
IonWorks/SyncroPatch	Current	384	L.
QPatch	Current	48	L.
Manual patch clamp	Current / potential	1	L. / T. / N. / S.
Scientifica Slice Master	Potential	4	S.
3Brain CMOS HD MEA	Potential	4.096	S.
Envision	pERK	384	N.
Brandel filtration unit	[^{3}H]-MK-801 binding	96	H.

Legend: L.: hNMDAR stable cell lines; T: hNMDAR transiently transfected cells; N: rat primary neurons; S: rodent brain slices; H: rat brain homogenates.

Objective

The objective of this poster is to illustrate few methodologies which can be applied to characterize various classes of NMDA receptor modulators in recombinant cell lines, using QPatch automated system.

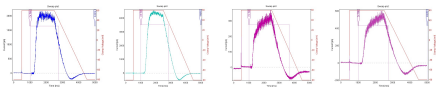
NMDAR Stable Cell Lines

CHO cell lines stably expressing diheteromeric NMDARs (hGluN1-hGluN2A, hGluN1-hGluN2B, hGluN1-hGluN2C or hGluN1-hGluN2D) were generated in Evotec. RefSeq protein accession numbers of NMDAR subunits are as indicated here below:

Subunit	Accession Number
hGluN1-1a	NP_015566
hGluN2A	NP_000824
hGluN2B	NP_000825
hGluN2C	NP_000826
hGluN2D	NP_000827

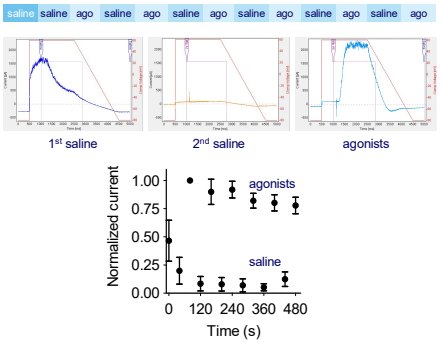
QPatch Protocol for Inward or Outward Current Measurement

- **Inward current measurement:** extracellular solution did not contain magnesium
- Fixed recording voltage at holding potential: -50 mV
- **Outward current measurement:** extracellular solution contained 1 mM $MgCl_2$
- Cells were stimulated from a holding potential of -80 mV to +60 mV with a 2 s step pulse, followed by a 2 s ramp to -80 mV, which allows visualization of Mg^{2+} block
- 100 µM glutamate plus 10 µM glycine were added during +60 mV step, after 500 ms from the depolarization (as indicated by pipette drawing)
- Steady state current amplitude could be measured at the end of the + 60 mV pulse
- Described voltage protocol could be applied every 40 seconds

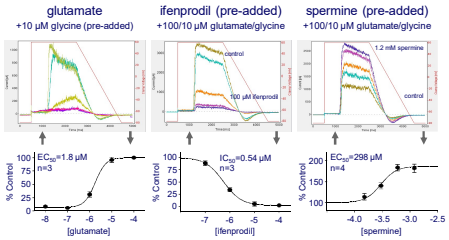


GluN1-GluN2B Current Stability in QPatch in Outward Current Protocol

- Saline or agonists (ago: 10 µM glutamate + 1 µM glycine) were added every 40 seconds, by QPatch protocol for outward current measurement using CHO cells expressing hGluN1-hGluN2B
- Agonists elicited current decreased down to an average 78% (n= 5) of its original value, 400 seconds after agonists first application



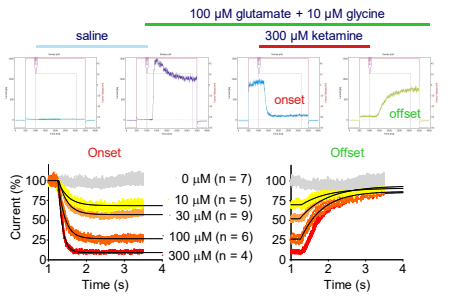
GluN1-GluN2B Pharmacology in QPatch



Control = 100 µM glutamate + 10 µM glycine. Data are mean ± SEM.

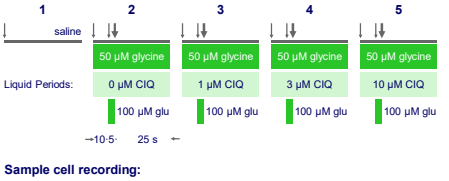
	hGluN1-hGluN2B	QPatch XC_{50} (µM)	manual patch XC_{50} (µM)
Comparison between QPatch and manual patch clamp data (average, n ≥ 3)	spermine	298	195
	ifenprodil	0.54	0.17
	glutamate	1.80	0.99
	glycine	0.11	0.13
	D-cycloserine	6.60	3.10
	ketamine	4.50	4.40

Onset/offset Kinetic Studies in QPatch: Ketamine on GluN1-GluN2B Receptor

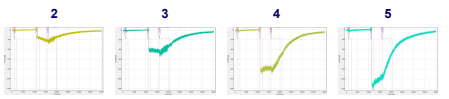


Ketamine conc. (µM)	Half-life$_{onset}$ (s)	Half-life$_{offset}$ (s)
10	0.18	0.48
30	0.16	0.51
100	0.13	0.40
300	0.08	0.37

Glutamate Deactivation Kinetic Studies in QPatch: CIQ on GluN1-GluN2D Receptor



Sample cell recording:



Liquid P.	CIQ conc. (µM)	Peak Current pA, (%)	Steady State Current pA, (%)	Tau, (s)	n
2	0	196±28 (100)	165±26 (100)	6.7±0.4	4
3	1	354±53 (180±6)	308±52 (186±5)	7.6±0.2	4
4	3	612±130 (303±24)	509±113 (299±22)	6.9±0.2	4
5	10	768±219 (367±60)	608±165 (348±45)	5.9±0.4	4

Conclusions

- CHO cell lines expressing human NMDA receptors composed of GluN1-1a subunit in combination with GluN2A, GluN2B, GluN2C, or GluN2D, have been generated
- Cell lines were profiled in automated QPatch. Excellent match of agonists, antagonists and modulators potencies is observed between manual patch clamp and automated QPatch data.
- On-set and off-set kinetic studies for NMDAR modulators were performed in QPatch: a good match of data was obtained with manual patch clamp data (not shown), for fast compounds such as ketamine
- Glutamate deactivation kinetic studies for NMDAR modulators were performed in QPatch. CIQ effect on GluN1-GluN2D cell line was shown as example, which was in perfect agreement with literature data (Mullasseril et al, 2010) obtained with manual patch-clamp.
- The four cell lines constitute an useful tool to profile compounds acting at various isoform of NMDARs