



NMDA RECEPTOR COMBINATIONS ARE DIFFERENTIALLY AFFECTED BY HIGH PRESSURE

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Introduction

Deep sea divers (90msw and more) experience High Pressure Neurological Syndrome (HPNS), which includes nausea, tremor, vision and auditory disturbances, convulsions, and loss of conciseness.



Deep Sea Professional Technical Diver. These saturation dives involve descending to depths down to 300 meters for days. This extreme environment may present great risks for the professional diver.

HPNS is associated with CNS hyperexcitability that may be attributed to augmented responses of the glutamatergic N-methyl-D-aspartate receptor (NMDAR).

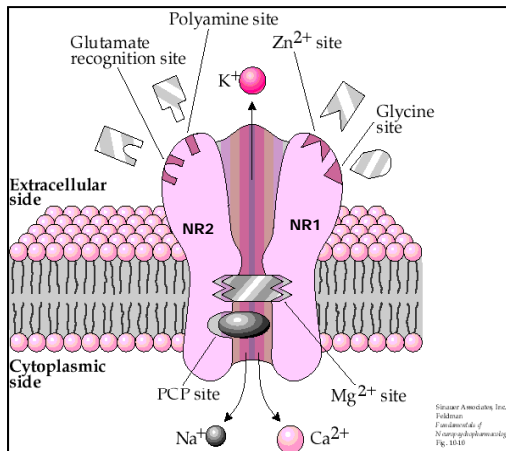


Fig. 1. NMDA receptor

A schematic presentation of major NMDAR subunits and binding sites. NMDAR is a hetero-tetrameric ion channel constituted of different combinations of 'NR1' (NR1-1a, -1b, etc.) with 'NR2' (NR2A, B, C, D) subunits.

We have recently reported that the NMDAR NR1-1a + NR2A combination current is augmented (Mor & Grossman, 2008) whereas NR1-1b + NR2A is depressed at 10.1 MPa.

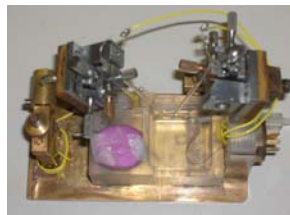
We now test the NR1-1a + NR2B and the NR1-1b + NR2B combinations under hyperbaric conditions.

Materials & Methods

Preparation: *Xenopus laevis* oocytes were prepared and injected with cRNA for co-expressions of rat NR1-1a or NR1-1b (5ng) with the rat NR2B subunit (5ng).

Electrophysiology: After 3-5 days individual oocytes were placed in a recording chamber and perfused with frog Ringer's solution without Mg^{2+} . Oocytes were voltage-clamped at -70mV.

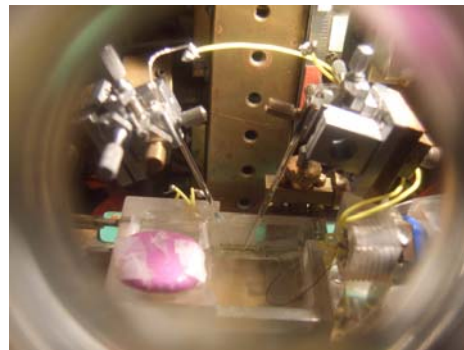
NMDAR currents were acquired under control (0.1-0.3MPa) and hyperbaric (10.1MPa, helium compressed) conditions.



Oocyte bath & electrodes



Compression chamber



Experimental setup in the compression chamber

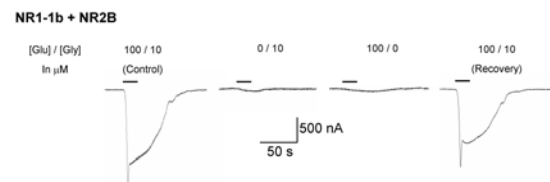


Fig 2. Confirmation of NMDAR expression in *Xenopus laevis* oocytes.

Activation of NMDAR requires simultaneous application of the co-agonists Glutamate (100 μ M) and Glycine (10 μ M). Wash time is 20 s (horizontal bar).

Past & Present Results

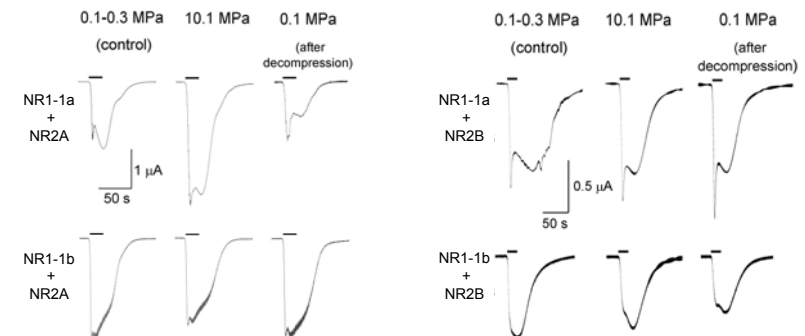


Fig 3. "NR2A" combinations are differentially affected by high pressure.

Single experiments are shown (0.1-0.3 MPa Vs. 10.1 MPa). High pressure increases and decreases NR1-1a + NR2A and NR1-1b + NR2A currents respectively.

Fig 4. "NR2B" combinations' are not affected by high pressure.

Single experiments are shown (0.1-0.3 MPa Vs. 10.1 MPa). These combinations seems to be "pressure resistant".

Subunits combination	Amplitude (nA) Mean \pm SE	Amplitude (nA) Mean \pm SE	Amplitude (% change) Mean \pm SE	n	p
	0.1-0.3 MPa	10.1 MPa	10.1/0.1 MPa		
NR1-1a + NR2A	648 \pm 141	925 \pm 232	+37.9 \pm 13.7	11	0.04
NR1-1a + NR2B	571 \pm 96	593 \pm 116	-1.4 \pm 7.2	12	0.6
NR1-1b + NR2A	1858 \pm 189	1561 \pm 201	-18.5 \pm 8.5	9	0.003
NR1-1b + NR2B	561 \pm 149	508 \pm 144	-6.5 \pm 6.4	10	0.115

Table 1. Statistical analysis of the NMDAR currents.

Statistical tests: paired t test (0.1-0.3 MPa Vs. 10.1 MPa).

Summary & Conclusions

NMDAR combinations are **differentially affected** by high pressure.

Mammalian hippocampal CA1 NMDAR combinations (mainly NR1-1a + NR2A, NR1-1a + NR2B) favor a **net response augmentation** (Mor & Grossman, 2006;2007;2008, recent oocyte experiments).

Following the work of Daniels et al. (1998), our data provide further evidence for selective NMDAR involvement in

- **Short term HPNS hyperexcitability.**
- Suspected **long term neurotoxicity** following repetitive exposure to high pressure.