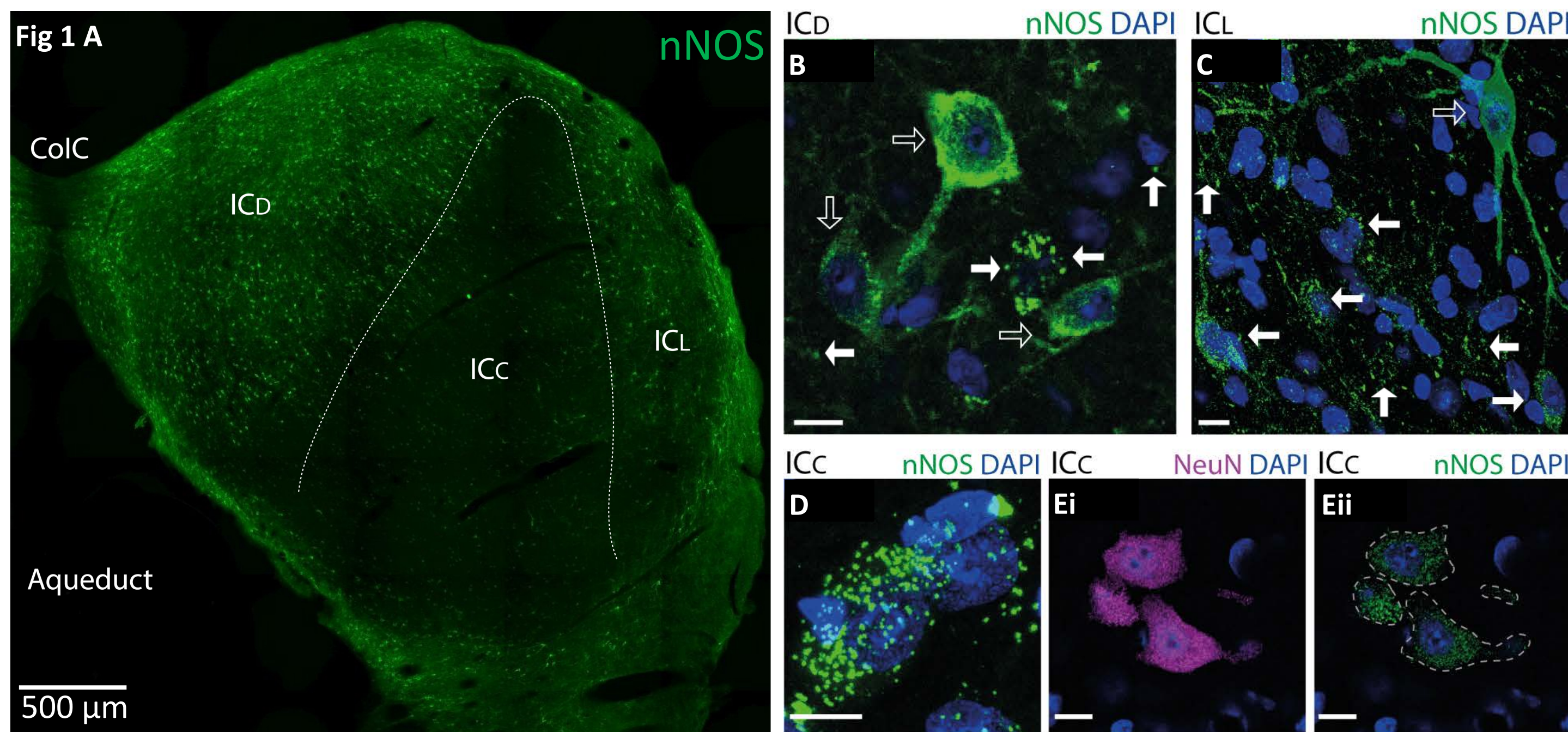


Expression of nNOS in the IC

Methods

- Four adult pigmented guinea pigs (3 female) were perfused with 4% PFA.
- Brains were harvested and 40 μm coronal sections were cut through the IC.
- Sections were labelled with combinations of primary antibodies targeting nNOS, soluble guanylate cyclase (sGC), the NMDA receptor (NMDA), the post synaptic density protein PSD95, GABA, and the neuronal cell body marker NeuN.
- Primary antibodies were visualised with fluorescent secondary antibodies using a confocal microscope (Nikon A+).

nNOS is expressed in all subdivisions of the IC



At low magnification, dense nNOS expression (green) is seen in the dorsal cortex (ICd) and the lateral cortex (ICl), but little is apparent in the central nucleus (ICc) (Fig 1A).

At higher magnification, in the ICd and ICl, neurons with nNOS labelling throughout their cytoplasm are abundant (open arrows Fig 1B & C).

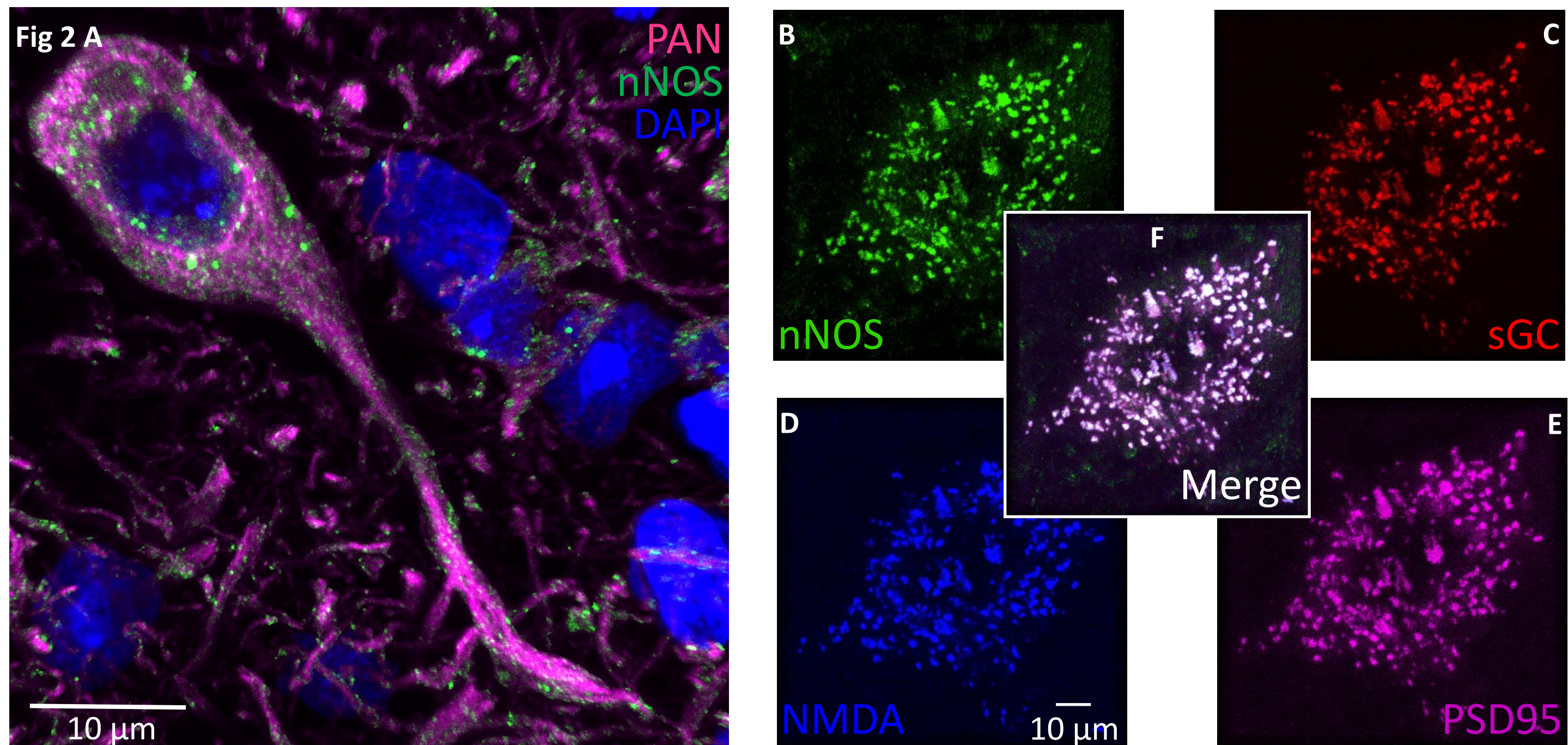
However, in ICd and ICl, amongst the 'nNOS filled cells', bright nNOS puncta can be seen (closed arrows Fig 1B & C).

Examination of the ICc revealed that nNOS puncta are also present in this region (Fig 1D).

Labelling for NeuN (magenta) revealed that all somata of neurons in the ICc apparently contain nNOS puncta (Fig 1Ei & Eii).

Double labelling with GABA showed that nNOS puncta occur in both GABAergic and non-GABAergic (presumed glutamatergic) neurons (data not shown).

nNOS puncta in ICc co-localise with sGC, NMDA & PSD95



Double labelling for nNOS (green) and Neurochrom (which labels all neuronal elements, magenta), showed that all nNOS puncta in the ICc are associated with neurones and that they occur on dendrites as well as somata (Fig 2A).

Quadruple fluorescent labelling (Fig 2F) in the ICc, showed that nNOS puncta (Fig 2B) co-localise with sGC (Fig 2C), the NMDA receptor (Fig 2D) and PSD95 (Fig 2E).

Introduction

The presence of neuronal nitric oxide synthase (nNOS) is one of the defining features of the inferior colliculus (IC). Indeed, nNOS labelling has been used to demarcate the IC cortices (Coote & Rees, 2008). However, the role nNOS plays in IC functioning and, by extension, in hearing is currently unknown.

nNOS is the enzyme catalysing the synthesis of nitric oxide (NO). NO readily diffuses through cell membranes allowing it to act both as an intra- and intercellular messenger.

Previous studies have reported nNOS to be absent from the central nucleus of the IC (ICc). Here we describe a punctate, form of nNOS expression in the ICc and reveal the contribution of NO to NMDA signalling in this region.

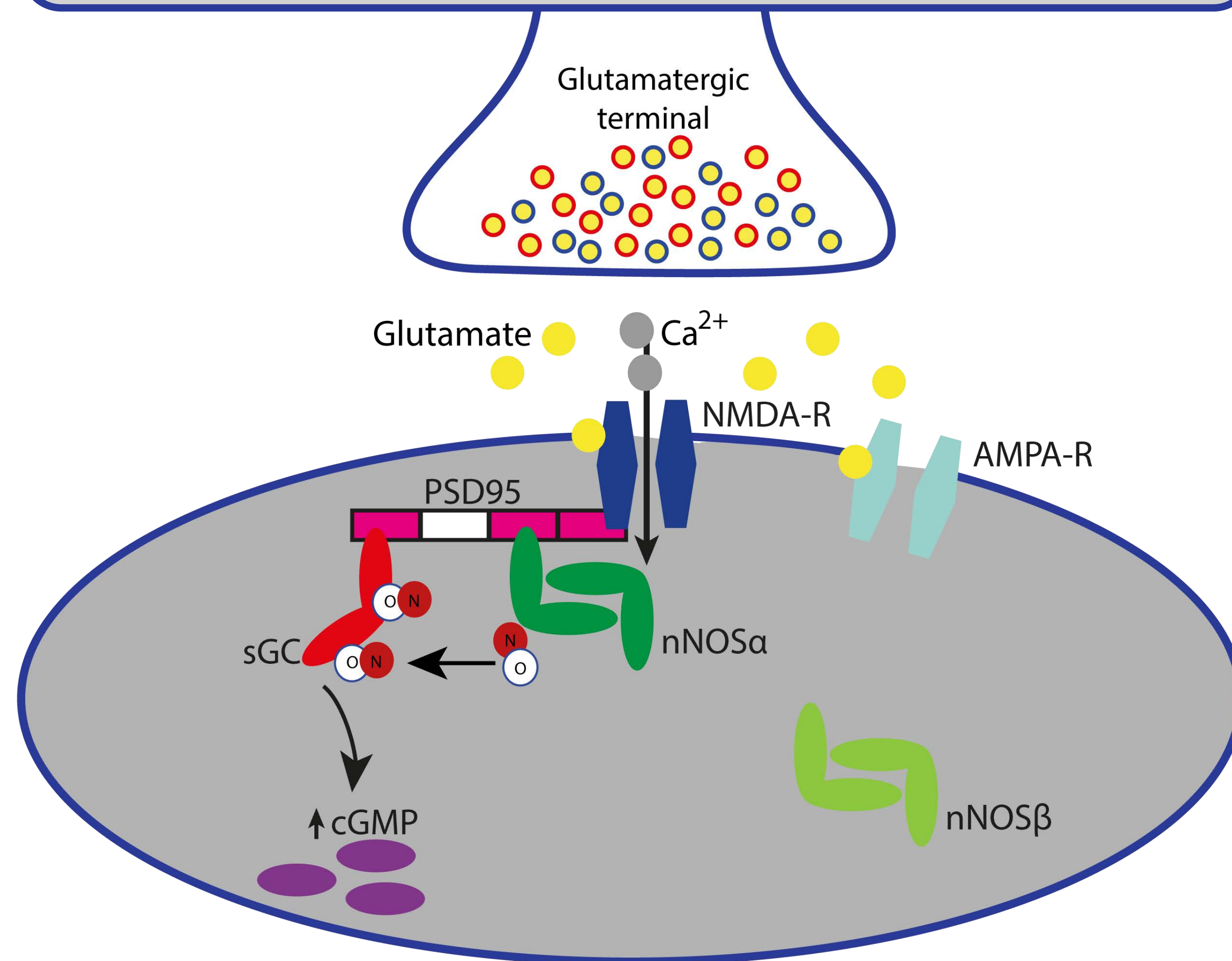


Fig 6. Proposed signalling pathway in which nNOS, the NMDA receptor and sGC bind to PSD95 to form a functional multi protein signalling complex.

Discussion

Here we show a novel, punctate, pattern of nNOS expression in the central nucleus of the IC. The puncta of nNOS co-localise with sGC, the NMDA receptor, and PSD95 suggesting the four proteins form a multi protein signalling complex (Fig 6).

Our functional studies revealed that the ability of NMDA receptor activation to modulate sound-driven activity in the IC is mediated via nNOS and sGC.

An upregulation of nNOS has been observed in other auditory structures in animal models of tinnitus (Zheng et al., 2006; see also Maxwell et al., Poster 815). Increases in neuronal excitability and firing that occur in the IC following acoustic trauma (Mulders and Robertson, 2009) or salicylate (Patel and Zhang, 2014) may involve the nNOS puncta and associated multi protein signalling discussed here.

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Function of nNOS in the IC

Methods

- Pigmented guinea pigs (350-650 g) were anaesthetised with urethane, fentanyl and midazolam.
- A microdialysis probe and a single shank 32-channel recording electrode (Neuronexus) were implanted in the right ICc (Fig 3).
- The microdialysis probe was perfused with artificial CSF (aCSF) or drugs targeting the nNOS/sGC pathway.
- Multiunit activity was recorded in response to pure tones (75 ms duration) at 256, 512, 1024, 2048 and 4096 Hz.
- Sound-evoked neuronal activity was recorded during baseline (aCSF) and drug conditions.

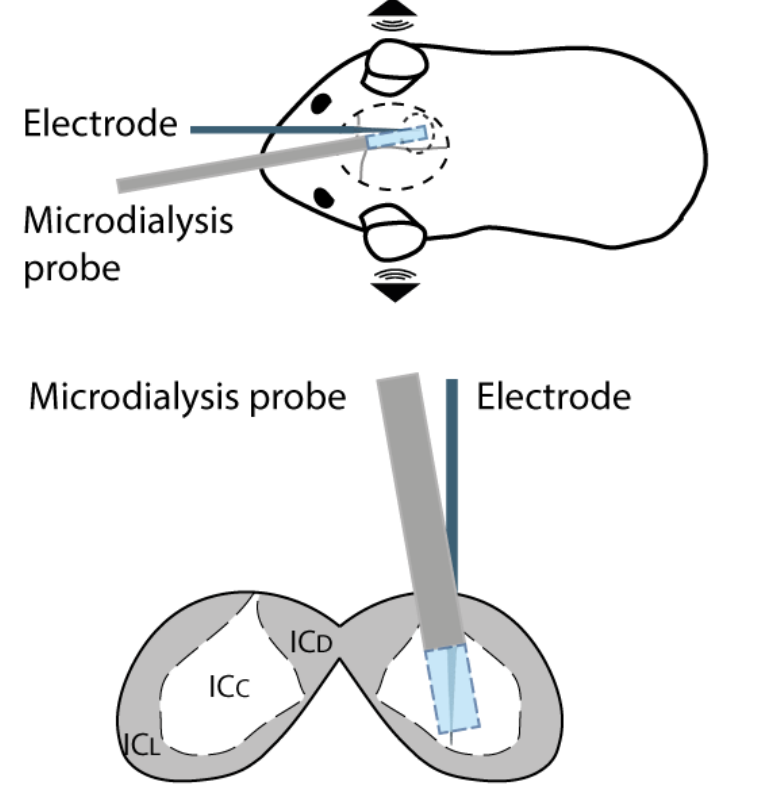


Fig 3. Schematic representation of implanted recording electrode and microdialysis probe in the IC.

NMDA increases sound driven activity via nNOS

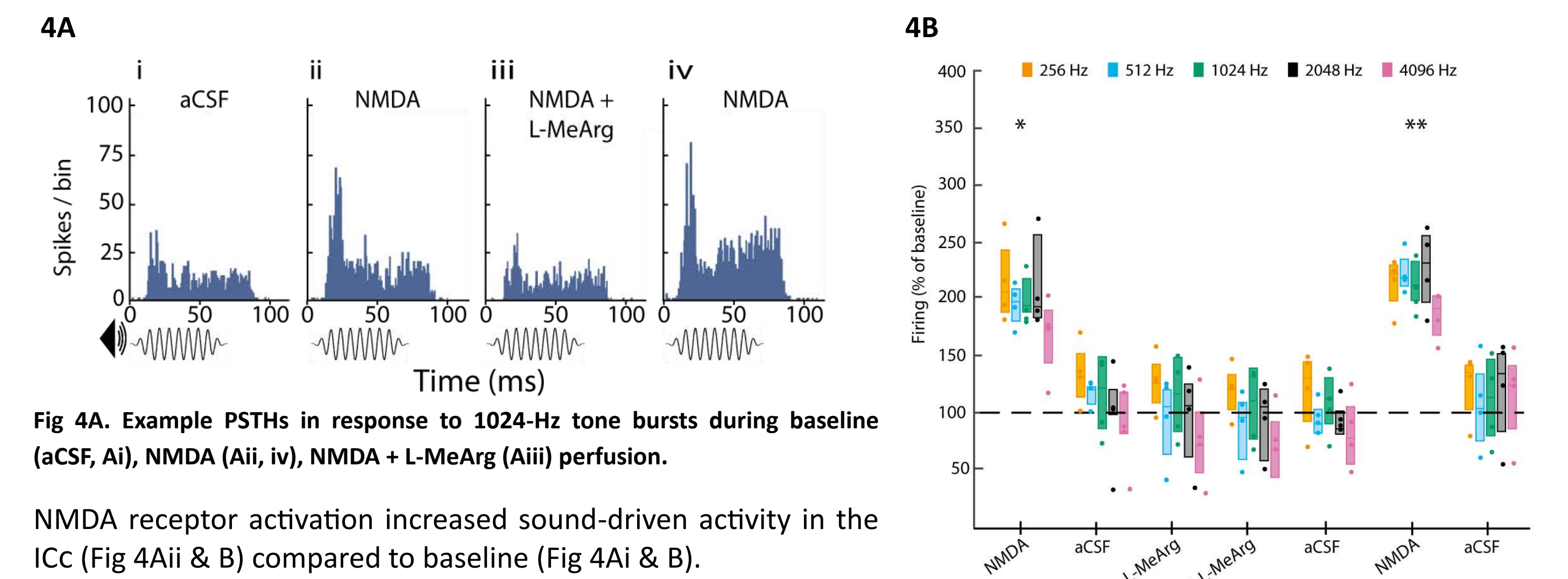


Fig 4A. Example PSTHs in response to 1024-Hz tone bursts during baseline (aCSF, Ai), NMDA (Aii, iv), NMDA + L-MeArg (Aiii) perfusion.

NMDA receptor activation increased sound-driven activity in the ICc (Fig 4Aii & B) compared to baseline (Fig 4Ai & B).

Co-perfusion of the nNOS inhibitor L-methyl arginine (L-MeArg) blocked the effect of NMDA (Fig 4Aiii & B).

The NMDA mediated increase was restored after L-MeArg was washed out (Fig 4Aiv & B).

NMDA increases sound driven activity via sGC

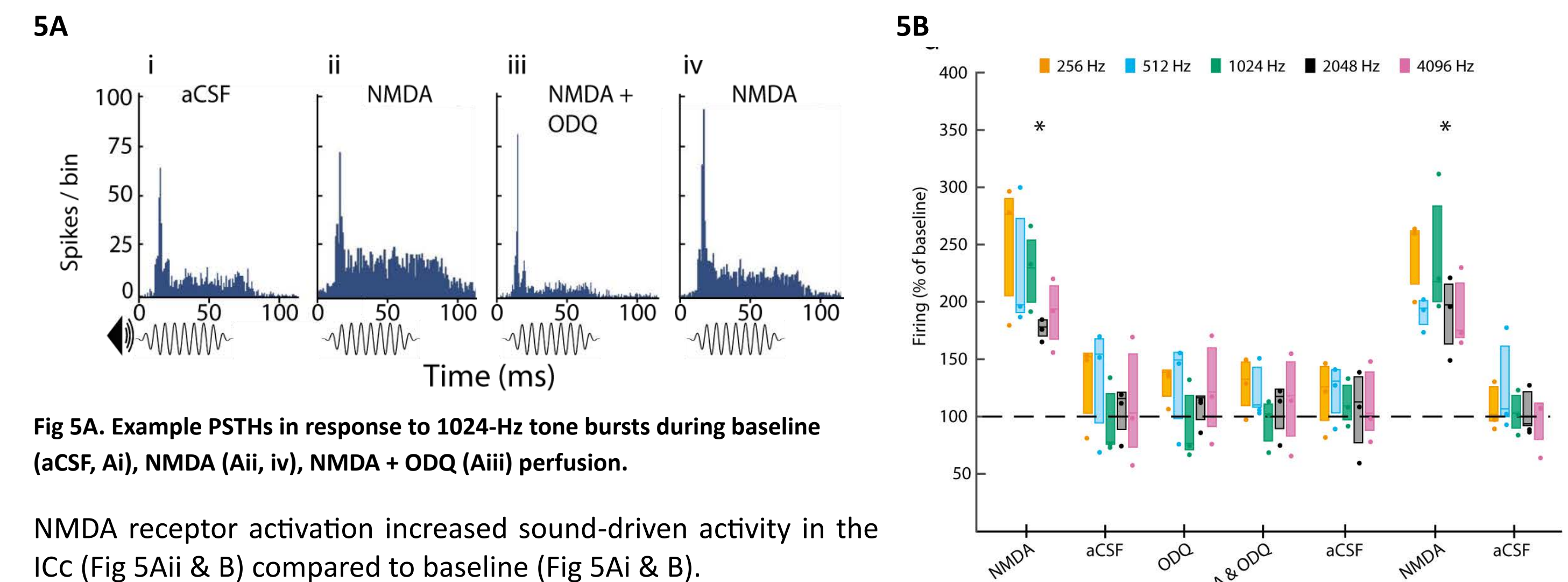


Fig 5A. Example PSTHs in response to 1024-Hz tone bursts during baseline (aCSF, Ai), NMDA (Aii, iv), NMDA + ODQ (Aiii) perfusion.

NMDA receptor activation increased sound-driven activity in the ICc (Fig 5Aii & B) compared to baseline (Fig 5Ai & B).

Co-perfusion of the sGC inhibitor ODQ blocked the response to NMDA (Fig 5Aiii & B).

The NMDA mediated increase was restored after ODQ was washed out (Fig 5Aiv & B).

References

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