Olfactory bulb evoked field potential by electrical stimulation of the olfactory epithelium in the anesthetized mouse: development of a potential Nav1.7 channel blocker assay

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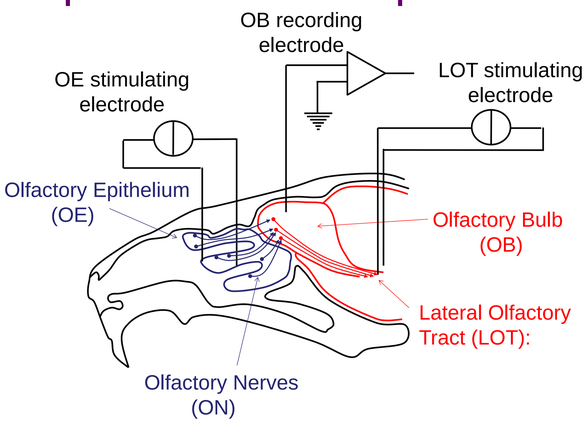
Introduction

In addition to a profound deficit in pain sensation, loss of function of Nav1.7 channel results in anosmia[1]. Nav1.7 is highly expressed in olfactory sensory neurons and their axons, but is not present in their post-synaptic target in the olfactory bulb (OB)[1,2]. Anosmia in Nav1.7 KO mice is caused by an inability of olfactory sensory neurons to transmit action potentials to their target in the OB: it is hypothesized that action potentials generated at the level of the soma in the olfactory epithelium (OE) are lost along their course to the OB[1,3].

A consequence of the above is that evoked field potential (EFP) generated in the OB in response to stimulation of the OE and their axons should constitute a Nav1.7 dependent response.
The objective of the present study was to explore the establishment of OB EFP in response to electrical stimulation (ES) of the OE in order to provide an in vivo Nav1.7 channel assay.

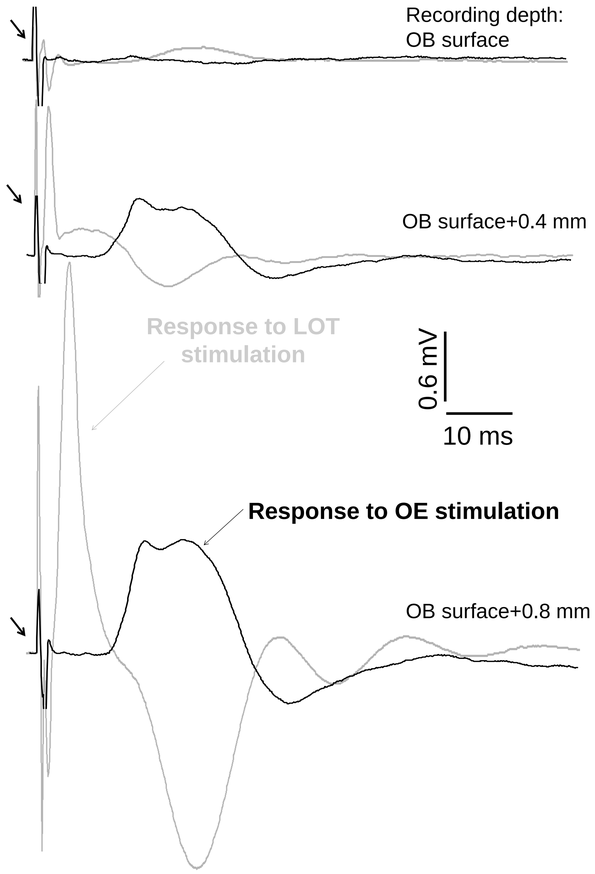
Experimental set up



- Olfactory sensory neurons in the OE (blue dot) project their axons (blue arrow) to the OB where they synapse with mitral and tufted cells. Axons of the mitral cells (red arrow and dot) en route to the piriform cortex constitute the LOT.
- OB EFP can be generated by electrical stimulation of the OE or the LOT, (orthodromic and antidromic stimulation, respectively). Orthodromic OB EFP is used here as a experimental control[4].

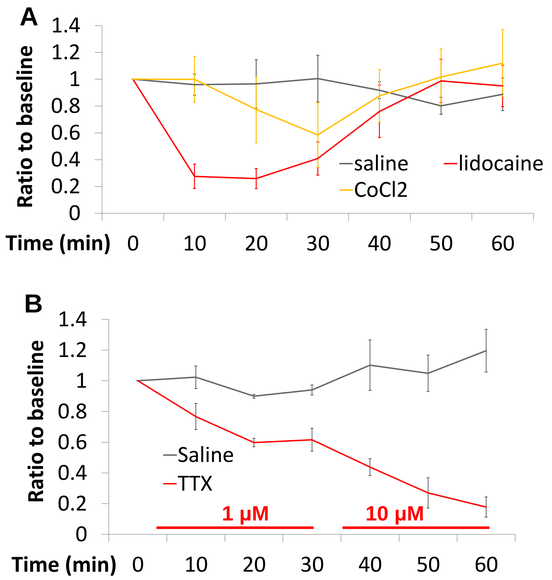
Results

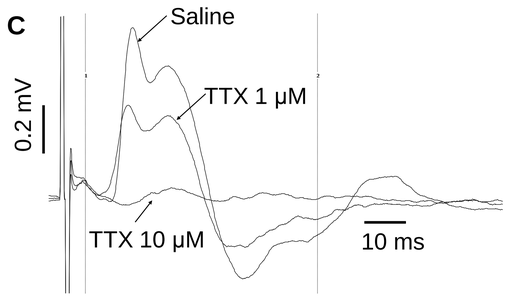
1. Example of antidromic and orthodromic EFP recordings in the OB.



- Typical EFP recordings obtained at different depths in the OB in response to electrical stimulation of the OE (black) or the LOT (grey). Arrow: stimulation artefact.
- OB EFP were unchanged by CoCl2 (10 mM) but abolished by lidocaine (2%) application on the OB. Amplitude of OB EFP was highly dependent on stimulating electrode placement (not shown).
- Note the low amplitude and long latency of orthodromic OB EFP (mean±s.e.m. of the positive deflection,10.7±0.4 ms, range 7.7-14.0 ms) compared with antidromic OB EFP.
- Orthodromic OB EFP were unchanged by neuromuscular blockade with gallamine (not shown).

2. Orthodromic EFP is sensitive to local application of lidocaine, $CoCl_2$ and TTX.





- The area under the curve of the rectified EFP (measured 0.8 mm below the surface) was computed before and after local application of drug or vehicle over the OB.
- A: 2% lidocaine was applied for 20 min and 10 mM $CoCl_2$ for 30 min before rinsing with saline.
- B: 1 and 10 µM TTX were applied successively for 30 min.
- C: Example of recording obtained with application of TTX (1 and 10 µM) as in B (area under the curve was computed between the dotted lines).
- Saline, n=3; lidocaine, $CoCl_2$, TTX 1 or 10 µM, n=4. Data expressed as mean±s.e.m. in A and B.

Methods

- Male C57BL6/J mice (20-30 g) were terminally anesthetized with urethane and the head placed in a stereotaxic frame.
- Holes were drilled over the top of the parietal, frontal and nasal bones to place recording and stimulating electrodes.
- EFPs were measured in the OB with a 5-10 MΩ tungsten electrode using a Neurolog set up (Amplification, NL100AK and NL104A, 1 K gain; Filter, NL125/126, 1 Hz-1KHz band pass).
- ES (biphasic square wave pulses) were performed with bipolar concentric electrodes for the LOT (0.2 ms, 0.1-1.0 mA; 0.22 mm rostral, 0.17 mm lateral and 6.0 mm deep from bregma) and bipolar wire electrodes (Pt or Ag-Ag/Cl) for the OE (0.5 ms, 0.1 to 2.0 mA).
- ES were conducted at 0.1 Hz and the average of 5 successive responses used for quantification.
- A well was made around the opening on the OB for local application of lidocaine, TTX and $CoCl_2$. The neuromuscular blocker gallamine thriethiodide was delivered i.v.

Conclusions

- The overall shape, latency and lidocaine sensitivity of the EFP recorded in response to electrical stimulation of the OE support that the EFP is effectively generated by the orthodromic activation of olfactory sensory neurons.
- The moderate effect of $CoCl_2$, and the relatively high concentration of TTX (10 µM) required to fully inhibit orthodromic EFP requires interpretation. It might be explained by the poor diffusion of these compounds through the OB or that only a fraction (40%) of the signal recorded is indeed due to post-synaptic current.
- The present model, once validated with a potent Nav1.7 channel blocker, should represent a powerful Nav1.7 screening assay.

References

1 Weiss et al, Nature, 2011, 472:186.
2 Ahn et al, Molecular Pain, 2011, 7:32.
3 Zufall et al, Arch Neurol, 2012, 69:1119.
4 Rall and Shepherd, J Neurophysiol, 1968, 31:884.