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RESEARCH ARTICLE

Expression of estrogen receptors α and β in the trigeminal mesencephalic nucleus of adult women and men

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ABSTRACT

Temporomandibular disorders are more prevalent in women than in men and phases of pain relate to the estrous cycle. Several studies described the location of estrogen receptors (ER) in the temporomandibular joint (TMJ), the masseteric muscles and cartilage, but it was unknown whether they are also expressed within the pseudounipolar neurons of the trigeminal mesencephalic nucleus, which receives direct sensory inputs from these structures. Therefore, we studied expression of ERα and ERβ protein in the trigeminal mesencephalic nucleus of ten human brains (five female/five male). Both receptors were uniformly expressed on neurons, but not other cell types within the target structure. Thus, sensory inputs from the TMJ and adjacent structures are likely to be modulated by estrogen at the level of the first sensory neuron which may underlie the well-known correlation of pain incidence and phases of the estrous cycle.

1. Introduction

The term temporomandibular joint (TMJ) syndrome defines a group of diseases of the temporomandibular joint and of the masseter muscles (Cairns, 2010; Warren and Fried, 2001). The etiopathogenesis of the temporomandibular disorder (TMD) seems to depend on many factors including trauma, anatomical variations, neuromuscular and psychosocial factors which all relate to a predisposition, release and maintenance of the disease (Cairns, 2010; Warren and Fried, 2001). It has been reported that temporomandibular joint pain affects woman 1.5–2 times more frequently than men, and that 80% of patients treated for TMD are women (Bush et al., 1993). Moreover, women were reported to exhibit more severe symptoms of TMD than men (Fillingim and Ness, 2000; Fillingim et al., 2009; Shinal and Fillingim, 2007).

Several observations suggest that sex hormones play an important role in the disease course of TMD: The prevalence is higher in women compared to men, but, strikingly, TMD-related pain often disappears after the menopause (Von Korf et al., 1988) when changes in estrogen levels are less pronounced (LeResche et al., 2003). Moreover, several lines of evidence indicate the impact of estrogen on cartilage and its metabolism (Breu et al., 2011; Claassen et al., 2001; Claassen et al., 2011; Kamiya et al., 2013; Schicht et al., 2014). In general, phases of pain usually develop after puberty and peak in the reproductive years (Meisler, 1999; Warren and Fried, 2001) with the highest prevalence appearing in women aged between 20 and 40 years and the lowest among children, adolescents and the elderly (Warren and Fried, 2001; Kuttila et al., 1998).

Moreover, oral contraceptives can alter symptoms in phases of pain. A clear correlation has been reported between TMD pain peak and the end of the menstruation, when the estrogen levels are lowest, but was also apparent in the middle of the cycle, when estrogen levels fluctuate (LeResche et al., 2003). Of note, women suffering from TMD symptoms also have a lower birth rate (Marbach et al., 1988; Warren and Fried, 2001).

While it is known that estrogens have a pain modulating effect in women (Mogil et al., 1993; Sternberg et al., 1995), the respective target structures of estrogens responsible for this effect are unknown. While a recent study reported the presence of estrogen receptors (ER) in the TMJ of the female baboon (Aufdemorte et al., 1986), their presence in sensory neurons of the TMJ has not been studied. In contrast to all other areas, where proprioceptive input is received by pseudounipolar neurons located in ganglia, the neurons receiving input from the temporomandibular region are located directly within the brain, namely within the mesencephalic trigeminal nucleus.

Estrogen acts primarily via two receptor proteins, ERα and ERβ, which upon hormone binding form mono and/or heterodimers and translocate into the nucleus where they bind to responsive elements in target gene promoters or alter gene expression via co-regulatory proteins (reviewed in Barnes et al., 2004) causing altered

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neuronal excitability (Bereiter and Okamoto, 2011; McEwen, 2001; Scharfman and MacLusky, 2006; Woolley, 2007). In this study, we tested whether the proprioceptive neurons of the TMJ or adjacent glial or vascular cells in the target area express ERα and/or β. To this end, mesencephalic trigeminal nuclei were dissected from 10 human brains (five male/five female).

2. Materials and methods

2.1. Tissues

Human brains of body donors and patients who died without signs of neurologic disease (five male/five female; aged between

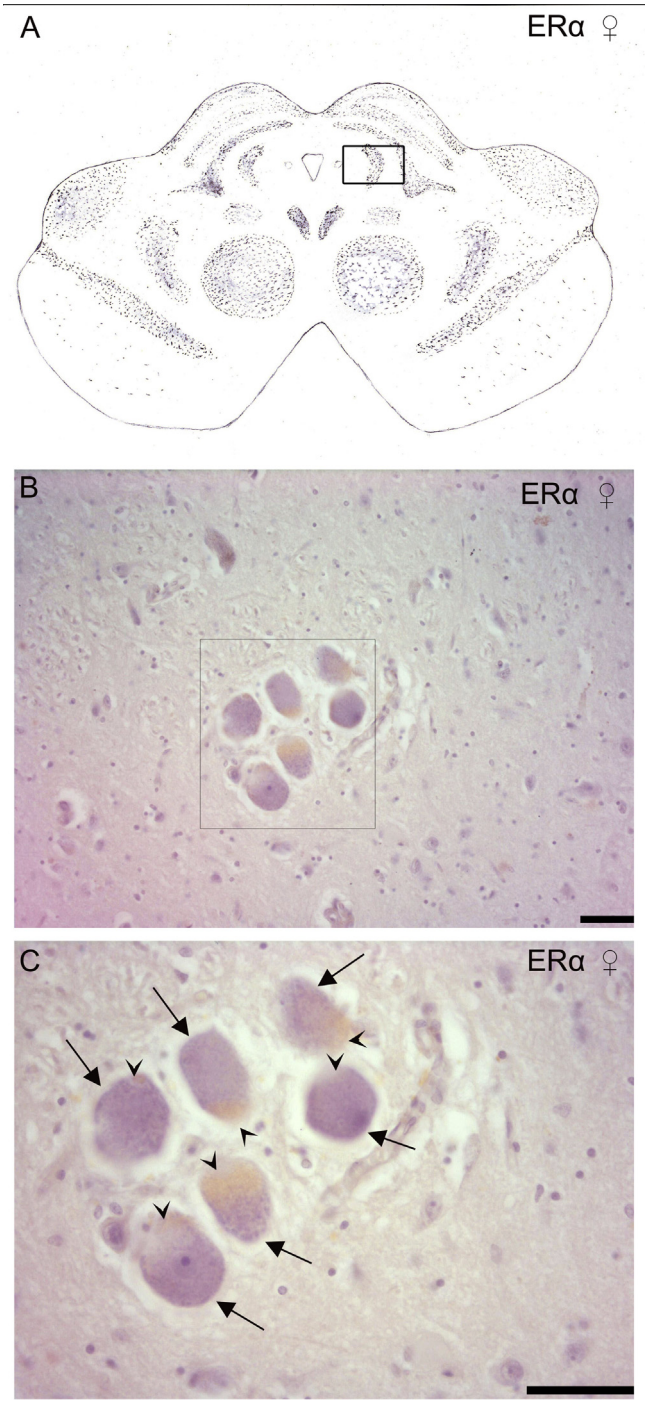

Fig. 1. Expression of ERα distributed in the region of the trigeminal mesencephalic nucleus in female brain
A: Schematic illustration of the midbrain with demarked region of the trigeminal mesencephalic nucleus.
B and C: ERα-positive neurons exhibiting polarized cytoplasmic and slight nuclear staining are shown in 14 μm paraffin sections of the female trigeminal mesencephalic nucleus. The area demarked in B is shown at high magnification in C. Scale bar: 50 μm. Labeled arrows: Pseudounipolar neurons. Labeled arrowheads: The positive immune staining.

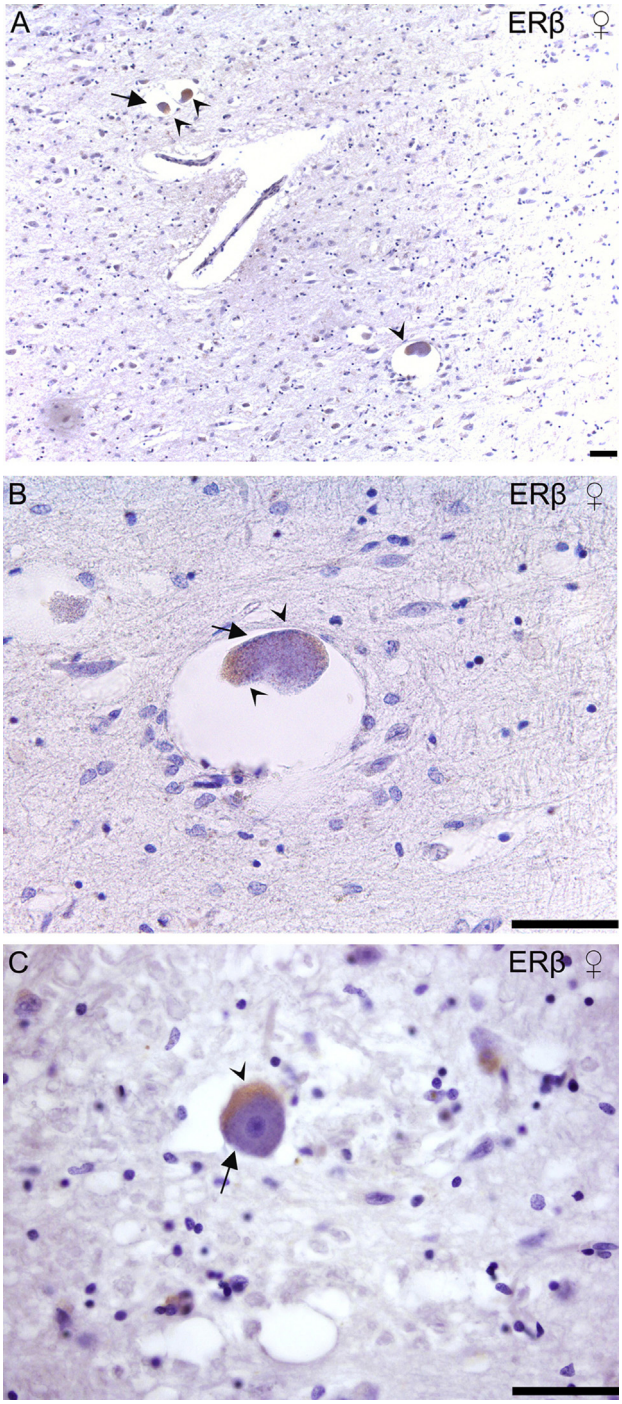

Fig. 2. Expression of ERβ distributed in the region of the trigeminal mesencephalic nucleus in female brain
A–C: ERβ-positive neurons are shown in 14 μm paraffin sections of the female trigeminal mesencephalic nucleus. Note the strong, polarized cytoplasmic and nuclear immune staining. Scale bar: 50 μm. Labeled arrows: Pseudounipolar neurons. Labeled arrowheads: The detectable positive immune staining.

45 and 88) and archival tissue samples of human normal uterus and breast cancer were used in this study. Body donors explicitly consented to the use of their tissues for research. All brain donors passed away in 2011. The brains were fixed in formalin for several days and then rinsed in water overnight. The brainstems were dissected, dehydrated in alcohol, and embedded in paraffin. Fourteen μm thick sections were cut using a microtome, placed on microscope slides, and then dried at 63° C for 30 min.

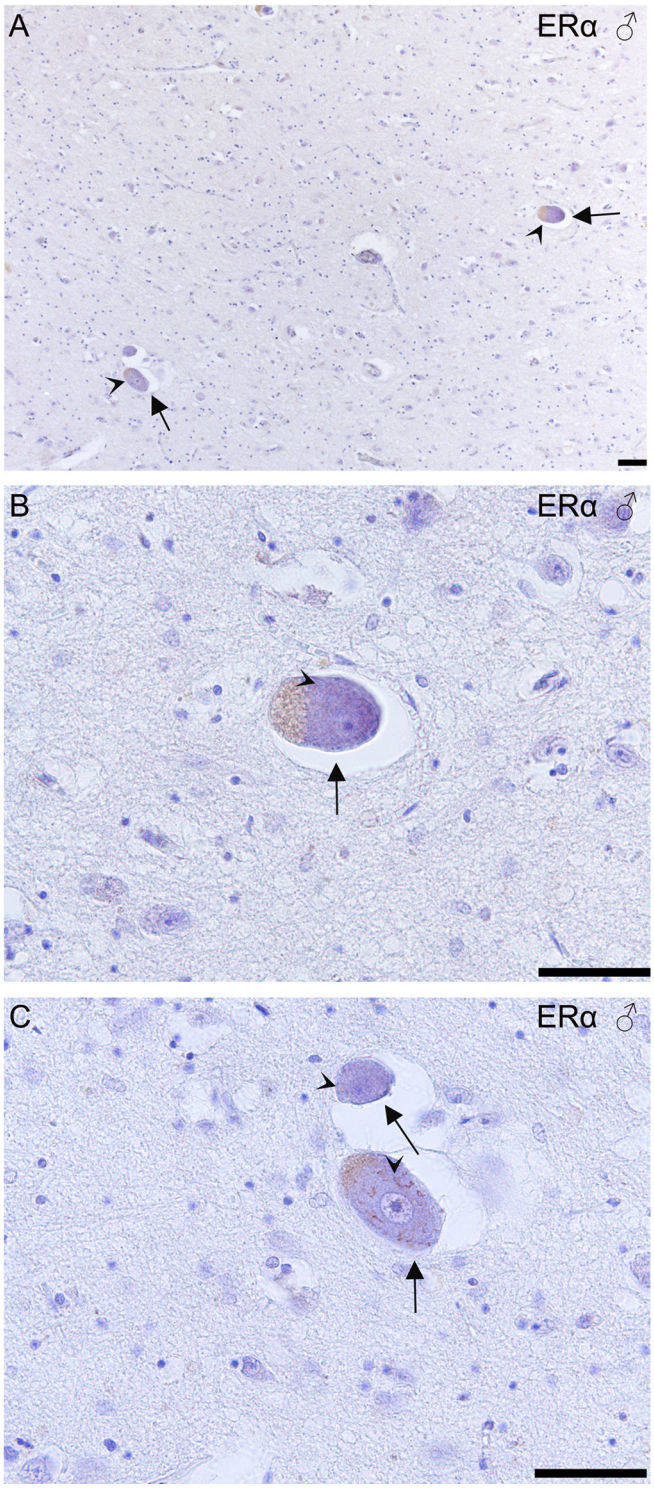

Fig. 3. Expression of ERα distributed in the region of the trigeminal mesencephalic nucleus in male brain
A–C: ERα-positive neurons are shown in 14 μm paraffin sections as depicted by the antibody and visualized with DAB in the male trigeminal mesencephalic nucleus. Note the strong nuclear immune staining. Scale bar: 50 μm. Labeled arrows: Pseudounipolar neurons. Labeled arrowheads: The clearly visible positive immune staining.

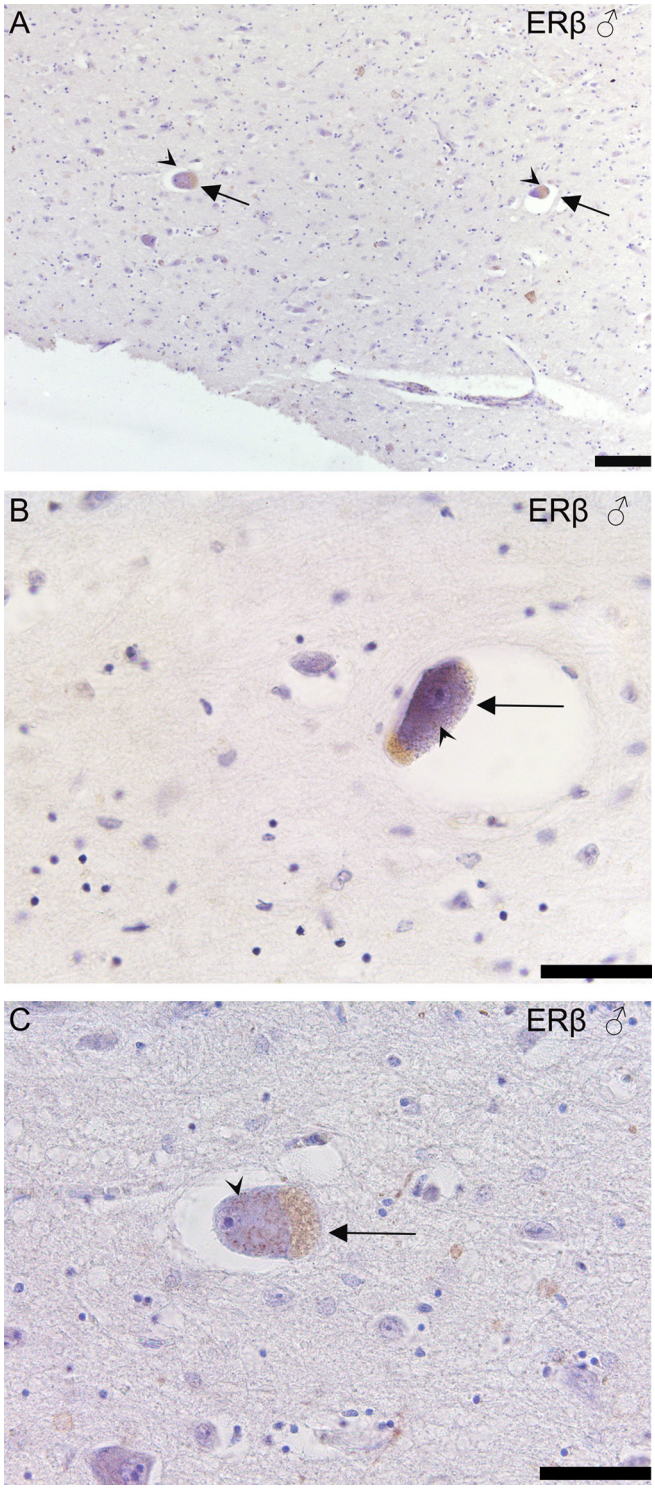

Fig. 4. Expression of ERβ distributed in the region of the trigeminal mesencephalic nucleus in male brain
A–C: ERβ-positive neurons exhibiting strong nuclear staining are shown in 14 μm paraffin sections as depicted by the antibody and visualized with DAB in the male trigeminal mesencephalic nucleus. Scale bar: 50 μm. Labeled arrows: Pseudounipolar neurons. Labeled arrowheads: The positive immune staining.

2.2. Immunohistochemistry

Paraffin sections were deparaffinized in xylene, rehydrated through a graded series of alcohol, and processed for antigen retrieval using incubation in citrate buffer (pH 6.0) for 5 min at 96° C. The sections were incubated in 0.5% H_2O_2 in methanol for 30 min at room temperature to quench endogenous peroxidase activity and then incubated in 0.5% Triton in phosphate buffered saline (PBS) for 30 min. Unspecific binding of primary antibodies was blocked by incubating the sections in normal goat serum (1:20) in 0.5% Triton in PBS for 30 min at room temperature. Sections were then incubated with anti-ERα (Dako, mouse monoclonal 1D5; 1:300) in 0.5% Bovine serum albumin (BSA), with anti-ERβ (Abcam, rabbit polyclonal ab3577; 1:500) in 0.5% BSA and 0.3% Triton in PBS, or with the corresponding buffer-only control overnight at 4° C. After washing, sections were incubated with the appropriate biotinylated secondary antibodies (diluted for ERα 1:100 and for ERβ 1:500) for 1 h at room temperature. After several washes in PBS, sections were incubated with Extra-Avidin-Peroxidase (Dako) for 1 h at room temperature. The sections were then washed with 0.05 M Tris and PBS for 30 min. The complex of bound primary and secondary antibody was visualized using exposure to

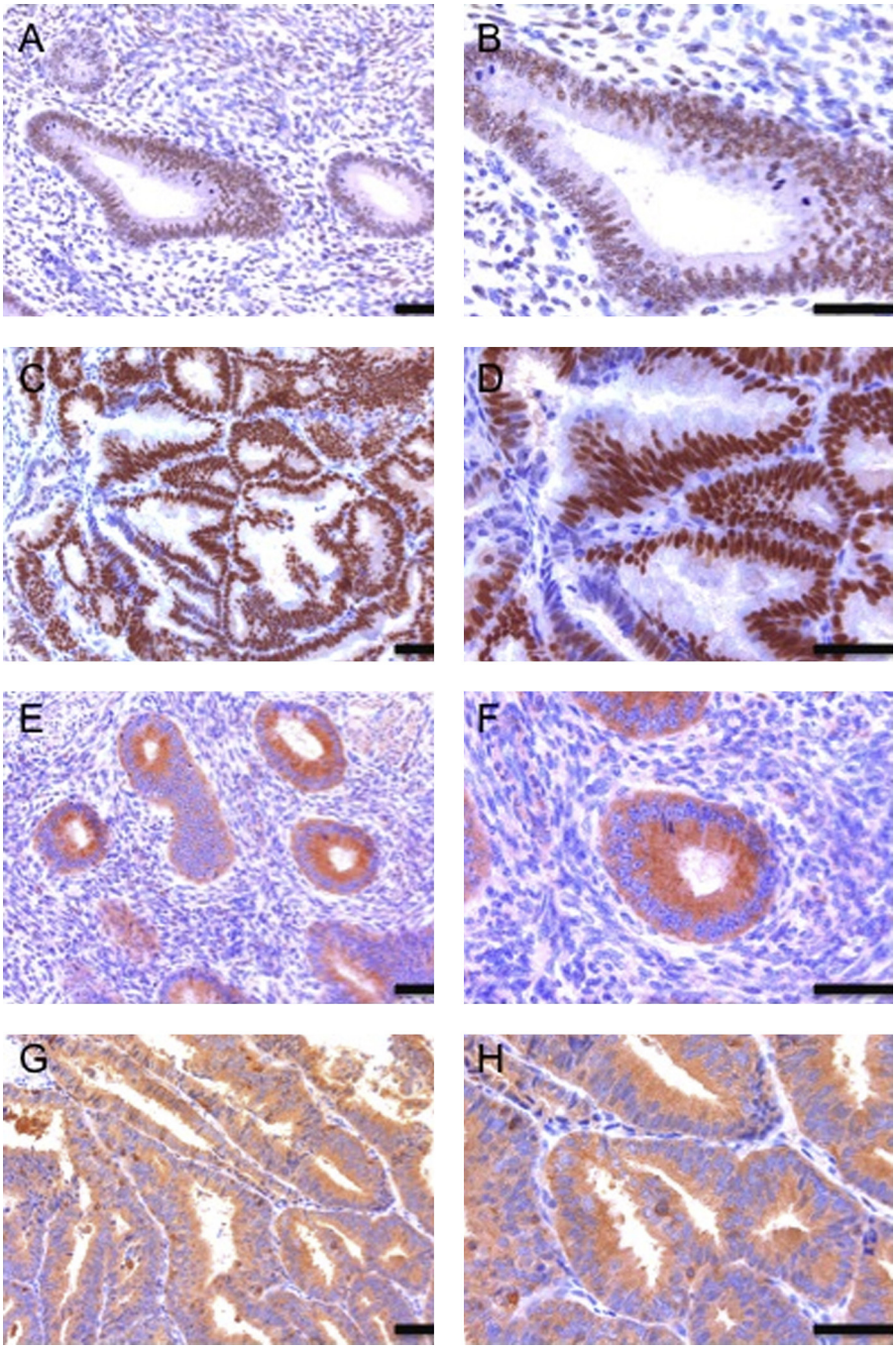

Fig. 5. Characterization of the ER antibodies used in the study by immunohistochemical staining of estrogen-sensitive tissue
A–D: The anti-ERα antibody 1D5 results in a clear nuclear staining of the glandular epithelium in the uterus (A and B) and of breast cancer cells (C and D).
E–H: Application of antiserum ab3577 suggests ERß immunoreactivity to be confined to the cytoplasm of the uterine epithelium (E and F) and of the breast cancer cells (G and H). Scale bar: 50 μm.

0.05 g Diaminobenzidine (DAB) and 50 μl H_2O_2 in 100 ml 0.05 M Tris. The sections were washed and lightly counterstained using hematoxylin, dehydrated through an ethanol series to xylene, and mounted. The reaction product appears as a brown punctuate stain.

2.3. Quantification

ERα or ERβ staining in the trigeminal mesencephalic nucleus was quantified in that the numbers of stained and unstained pseudounipolar neurones were determined for each receptor protein in 6 to 7 non-overlapping serial sections of each individual brain and then averaged for all brains in the study regardless of gender. The mean values are reported as percentage of stained and unstained cells and standard deviations are given.

3. Results

3.1. Expression of ERα

The trigeminal mesencephalic nucleus was easy to identify, since it is the only brain area where pseudounipolar neurons can be found (Figs. 1–4). The percentage of pseudounipolar neurons encountered with ERα staining in the 14 μm paraffin sections was 92 ± 0.95%. In female trigeminal mesencephalic nuclei virtually all neurons exhibited strong polarized cytoplasmic and slighter nuclear staining for ERα, while adjacent glial cells remained unstained (Fig. 1). The same pattern was observed in the trigeminal mesencephalic nucleus of males (Fig. 3). There was no evident difference between female and male nuclei neither in regard to the strength of the immune reaction nor to the amount of immune positive neurons. When uterine tissue and breast cancer tissue were selected as estrogen receptor expressing control tissue and stained with the anti-ERα antibody used in the study an identical nuclear staining pattern was obtained as in the midbrain sections. Furthermore omission of the primary antibody from the staining schedule resulted in unstained sections (Fig. 6).

3.2. Expression of ERβ

ERβ immune reactivity was also observed in most, i.e. 91 ± 0.96%, of the large pseudounipolar neurons of the trigeminal mesencephalic nucleus, while glial cells of the same area did not bind the antiserum. There was no evident difference between female and male nuclei either in regard to the amount of stained cells, the strength of the immune reaction or to the amount of immune positive neurons, Fig. 2 and Fig. 4, respectively. When the above reported estrogen sensitive tissues were stained with the same anti-ERß antiserum as the brain sections, an identical pattern of cytoplasmic ERß receptor immunoreactivity was produced as in the midbrain sections (Fig. 5). Again, omission of the primary antibody from the staining schedule resulted in unstained sections (Fig. 6).

4. Discussion

Many studies have reported a clear-cut correlation between sex hormones/estrous cycle and the appearance of phases of pain during TMJ-disorders (Abubaker et al., 1993; Aufdemorte et al., 1986; Fillingim et al., 2009; LeResche et al., 2003; Ribeiro-DaSilva et al., 2009; Warren and Fried, 2001). Here, we addressed the question of whether the sensitive neurons of the human trigeminal mesencephalic nucleus express estrogen receptors. To this end, five male and five female brains have been studied. We found that far more than 90% of the pseudounipolar neurons in the trigeminal mesencephalic nucleus expressed ERα and ERβ in both, female and male brains. Thus, it appears that respective brain regions receiving sensory inputs from the TMJ can sense estrogen. This is in addition to the already established belief that TMJ-tissue itself can sense estrogen (Aufdemorte et al., 1986; Abubaker et al., 1993; Kamiya et al. 2013; Orajarvi et al., 2011; Wang et al., 2008). Estrogen-receptive sensory neurons are in line with the recent demonstration of ER in rodent dorsal root ganglia (Sohrabji et al., 1994; Papka et al., 1997).

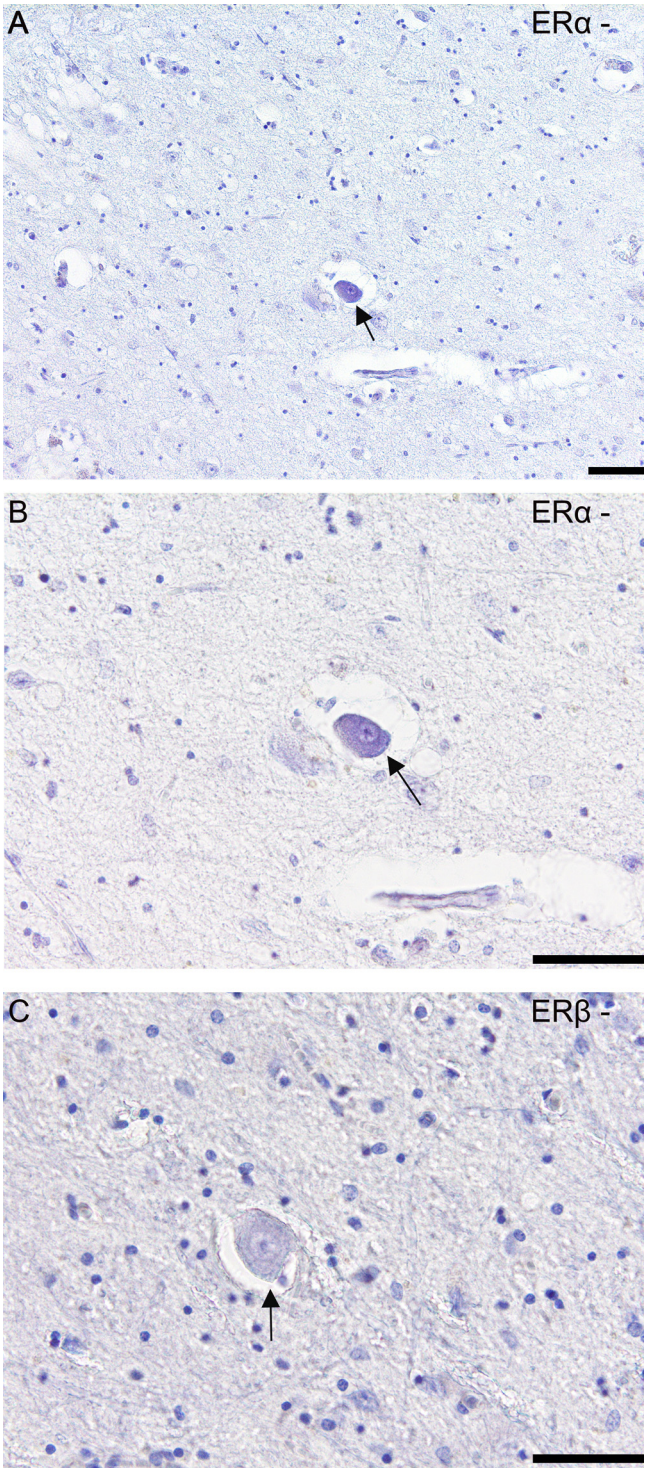

Fig. 6. Specificity controls of the secondary antibody
A–C: No immunoreactivity is seen in the mesencephalic nucleus of the trigeminal nerve, when primary antibody 1D1 against ERα (A and B) or antiserum ab3577 (C) is omitted in the staining schedule of the 14 μm thick brain sections. Labeled arrows point to unstained pseudounipolar neurons. Scale bar: 50 μm.

Recently, Yu et al. (2012) have shown that ovarectomy decreased the facial mechanical pain threshold in female rats and enhanced mRNA and protein expression of P2X3 receptors in trigeminal ganglia which are strongly involved in pain signaling (Ford, 2012). On the same path, Hu et al. (2011) demonstrated that 17β receptors regulate the gene expression of voltage-gated sodium channel proteins. Voltage-gated sodium channel protein (Nav) 1.1, Nav1.7, Nav1.8, and Nav1.9 were elevated in mice knocked out for ERα or ERβ, while Nav1.6 was decreased in ERα knock-out mice only. In fact, expression of ATP-gated cation channels is found on proprioceptive neurons in the trigeminal mesencephalic nucleus and appears to be functional in neurotransmission to the trigeminal mesencephalic motor nucleus (Khakh and Henderson, 1998).

While these and similar data (reviewed in Bereiter and Okamoto, 2011) provide a molecular basis for estrogen-induced alterations in neural transmission, they do not explain the susceptibility of individuals who suffer from TMJ-disorders. It is therefore of high interest that an association between polymorphism in ER-coding genes and pain susceptibility has been found in female TMJ-patients (Kang et al., 2007; Ribeiro-DaSilva et al., 2009). Such polymorphism could impact on the wiring between different brain areas involved in the generation of pain, but may also change the threshold of individual neurons to create action potentials. Of note, the GABAergic tone is responsive to estrogen (Brack and Lovick, 2007) and lack of GABAergic inhibition could well underlie increased susceptibility to pain. Moreover, it has been shown that estrogen influences synaptic plasticity by an increase in spines at apical dendrites of pyramidal neurons (Gould et al., 1990; Woolley et al., 1990; Kretz et al., 2004; Prange-Kiel and Rune, 2006; Yun et al., 2007) and may have neuroprotective effects (Garcia-Segura et al., 2001; Fester et al., 2011).

Whatever turns out to be the major site of estrogen-induced susceptibility to TMJ-related pain, we have shown in this paper that the proprioceptive neurons of the TMJ region in the human trigeminal mesencephalic nucleus possess both ERα and ERβ, and, thus, can be direct targets of estrogen-induced alterations in pain-signalling pathways.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aanat.2014.06.003.

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