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Endopiriform nucleus connectivities: the implications for epileptogenesis and epilepsy

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Several anterograde and retrograde tracing studies have provided detailed information on the afferent and efferent projections as well as the intrinsic connectivities of the endopiriform nucleus (EN). Here, we summarise EN connectional data and the principles of their organisation and discuss the role they may play in the development and spread of epileptic seizures.

Key words: olfaction, temporal lobe, seizure

The endopiriform nucleus (EN) is composed of densely packed dark multipolar cells that underlie the whole extent of the piriform cortex [2, 22]. The exact physiological role of the nucleus is still not known. However, it receives extensive reciprocal projections from the olfactory cortices [2, 24, 28, 42] and amygdaloid regions involved in the modulation of behaviour that is dependent on the convergence of olfactory cues [5, 23, 25, 26]. On the basis of these anatomical data, as well as electrophysiological and pharmacological evidence, Behan and Haberly [2] suggested that EN may influence the formation of olfactory memories or even emotional learning by linking odours with context information. This hypothesis is also supported by the findings of Fu et al. [14], who described the convergence of olfactory and gustatory connections onto EN neurons. Thus the EN may also be involved in the modulation of the mechanisms responsible for food selection and emotional reaction to chemical senses.

Several studies have also implied that EN plays an important role in epileptogenesis and epilepsy. In various models of epilepsy EN has been shown to be crucial for seizure induction and spread [9, 11, 38, 45]. The aim of this review is thus to summarise the afferent and efferent projections of EN and to discuss their possible role in the development of epilepsy (epileptogenesis) and, later on, in the spread of seizures.

ANATOMICAL INTERCONNECTIVITY OF THE ENDOPIRIFORM NUCLEUS

The most prominent afferent and efferent projections of EN have been summarised in Figure 1.

Intrinsic connectivities of the endopiriform nucleus

The endopiriform nucleus possesses heavy intrinsic projections through the whole rostrocaudal extent of the structure. The existence of these intrinsic projections was suggested by Hoffman and Haberly [16], who observed that the principal cells in EN excite other neurons within the structure via glutamergic synapses. This indirect evidence was confirmed by an elegant study by Behan and Haberly [2], who injected anterograde tracer (PHA-L, *Phaseolus vulgaris leucoagglutinin*) into the rostral, middle and caudal parts of EN and observed labelled axons extending for long distances in both rostral and caudal directions from each injection site.

Efferent projection of the endopiriform nucleus

The most detailed and systematic study of EN efferents has been performed by Behan and Haberly [2].

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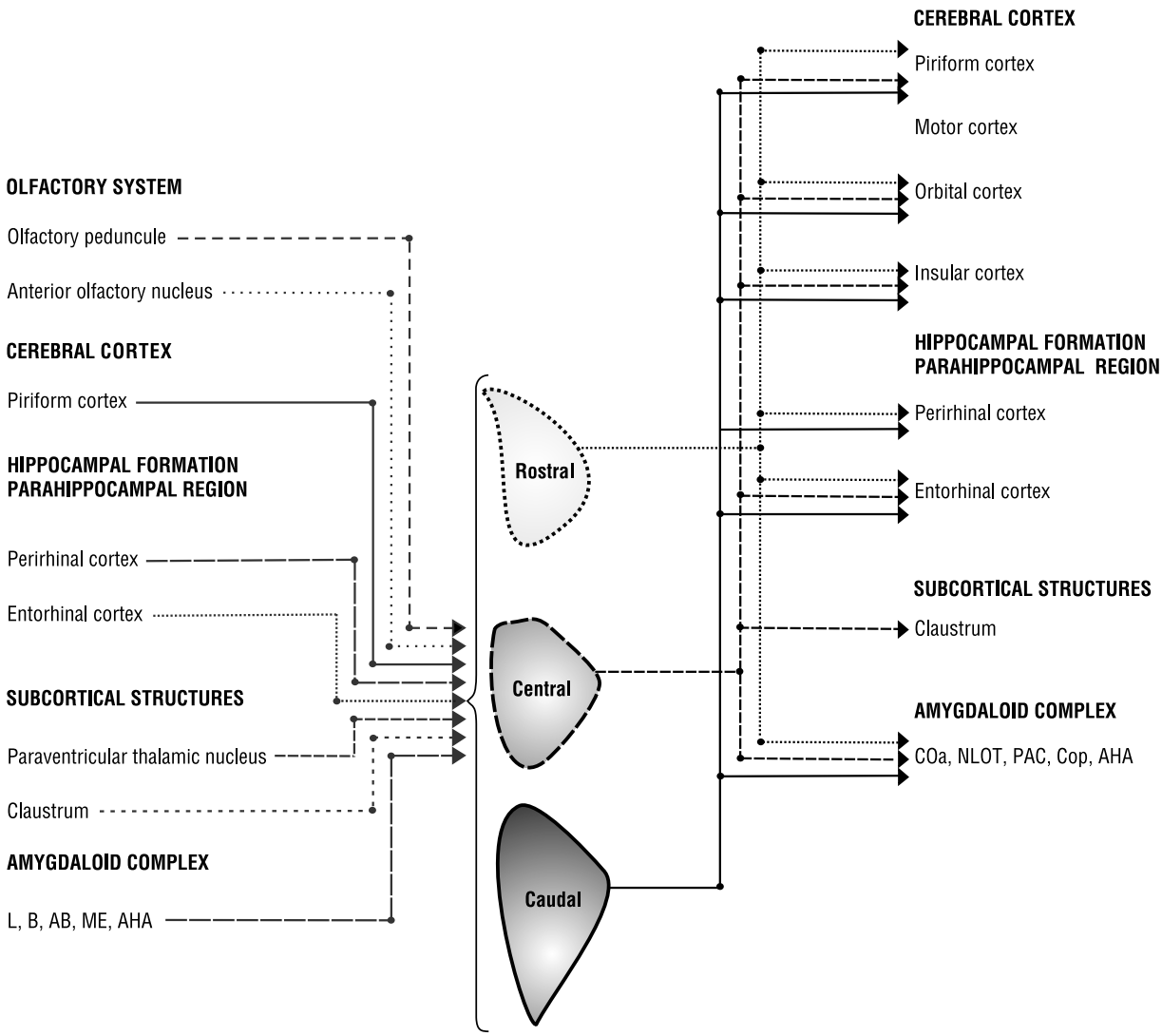

Figure 1. Schematic drawing summarising the heaviest afferent and efferent connections of the endopiriform nucleus. As a result of the less detailed information about afferent projections of the EN (left side of the scheme) the distribution of the projections within the rostrocaudal extent of the structure is not specified.

Their data, from the anterograde tracer (PHA-L) study, are generally in agreement with previously available findings [4, 15, 21]. Behan and Haberly [2] revealed that EN sent the most substantial projections to the cortical areas. Projections to the nuclear type of structure were, apart from the heavy intrinsic projections to EN itself, rather sparse. These cortical projections are highly distributed spatially. Thus the axons of neurons from a small region of EN extend over a broad region of targeted areas, and efferents from the anterior, middle and rostral parts of EN overlap within the area. There was also little difference in the laminar organisation of the connectivities. In general, the efferents were observed in all layers of the targeted cortical structures.

The endopiriform nucleus sent moderate to heavy projections to both the anterior and posterior piriform cortex, areas 35 and 36 of the perirhinal cortex and all 6 divisions, according to the nomenclature introduced by Insausti et al. [19], of the entorhinal cortex [2, 13]. It also projects to the lateral and ventrolateral divisions of the orbital cortex and agranular insular cortex [2]. The anterior olfactory nucleus, however, received the most extensive projections, predominantly from the anterior and middle parts of EN [2]. Within the amygdaloid complex the heaviest projections from all three parts of EN were also directed to the cortical structures: the nucleus of the lateral olfactory tract, the anterior and posterior cortical nuclei, all divisions (rostral, central and caudal)

of the medial nucleus and three subfields (PAC, PACm and PACs) of the periamygdaloid cortex. The projections to the lateral, basal, accessory basal and central nuclei and anterior amygdaloid nuclei are much lighter. It remains unclear weather the amygdalohippocampal area and the bed nucleus of the accessory olfactory tract receives any projections from EN [2]. The nomenclature used for partitioning of the amygdaloid complex was originally described by Price et al. [31] and modified by Pitkänen [30]. The projections to the hippocampal formation were very sparse and composed of few labelled fibres in the subiculum, presubiculum and parasubiculum, as well as the dentate gyrus and CA1 and CA3 regions of the hippocampus proper.

Afferent projections of the endopiriform nucleus

There is less detailed information about afferent projections of EN. The available data indicate that almost all EN connections with the olfactory-related areas are reciprocal. The heaviest afferent projections are derived from the piriform cortex [22, 28, 35]. A study by Schwabe et al. [35] revealed that PHA-L injections placed in the rostral, central and posterior aspects of the piriform cortex resulted in numerous fibres labelled within EN. The entorhinal cortex also sends projections to EN [19, 39, 46]. According to PHA-L study by Insausti et al. [19], projections from the medial aspect of the entorhinal cortex (CE field of the entorhinal cortex in their original description) are heavier than projections from the lateral aspect (fields DLE and DIE). The olfactory peduncle and anterior olfactory nucleus also project there [22]. A study by Shi and Cassell [37] described projections from the dorsal bank of the rhinal sulcus, which may correspond to area 35 of the perirhinal cortex. The region corresponding to area 36 does not seem to project to EN [19]. The infralimbic and dysgranular parietal insular cortices sent light projections to EN [18, 36].

The amygdaloid complex projections are also reciprocal. These are generally directed to the caudal two thirds of EN and are lighter in their density then projections from EN [25]. Only the medial nucleus and lateral division of the amygdalohippocampal area sent moderate projections to EP [5, 25]. In addition, light projections from the deep amygdaloid nuclei, such as lateral, basal and accessory basal nuclei, were also observed. Interestingly, the basal and accessory basal nuclei also projected to the anterior aspect of EN. All amygdaloid projections are unilateral.

Moga et al. [27], who injected PHA-L into the paraventricular thalamic nucleus, observed light staining in EN.

Contralateral projections

Anatomical evidence does not indicate any efferent projections from EN to the structures in the contralateral hemisphere of the brain [2, 15]. In contrast, EN receives afferents from both hemispheres. These projections, however, seem to be derived from cortical areas rather then nuclear structures. They are also lighter in density then ipsilateral projections. Contralateral projections from the entire piriform cortex [35] and infralimbic cortex [18] have also been confirmed. On the other hand, the cortical areas included in the amygdaloid complex (such as the periamygdaloid cortex) do not give any projections to the contralateral EN [25].

THE ENDOPIRIFORM NUCLEUS IN EPILEPTOGENESIS AND EPILEPSY

The endopiriform nucleus started to attract the attention of epileptologists when several *in vivo* and *in vitro* studies demonstrated that epileptiform activity in several locations in the limbic system spreads to the olfactory cortex. For example, studies by Piredda and Gale [29] and Sperber et al. [40] demonstrated that the anterior aspect of EN has a lower threshold for convulsant drug-evoked seizures in the rat than the remaining structures in the cerebral hemisphere. Stevens et al. [41] showed that an infusion of vigabatrine (a GABA transaminase inhibitor and antiepileptic drug) into this region increased the seizure threshold in rats. In line with this, there are observations from the kindling model of epilepsy in which periodic application of an initially subconvulsive stimulus leads to first limbic and then generalised tonic-clonic seizures. These revealed that EN has the lowest threshold and the highest kindling rate of the brain structures. Moreover, these epileptiform events could occur in EN alone, but in the piriform cortex they were only observed following the onset of epileptiform activity in EN [10, 11, 16, 17]. These features of EN may be explained by differences in intrinsic membrane properties, namely a more depolarised resting potential, lower spike threshold and higher input resistance of EN cells compared to layer II of the piriform cortex [43, 44]. Specific properties of the potassium and calcium currents may also partially explain the susceptibility of EN to seizure induction and expression [1, 3]. On the other hand, the suppression of activity in this region blocks seizure

spread from kindled amygdala [41]. Another interesting observation comes from the ischaemia model [20]. Pharmacological blockade of non-N-methyl-D--aspartate (NMDA) receptors in EN prevented hippocampal cell death. This indicates that EN not only plays an important role in seizure generation and propagation but may also modulate neuronal survival.

According to previous data, other studies have demonstrated that EN is affected by the epileptic process. Systemic or local administration of kainic acid and pilocarpine are commonly used in epilepsy modelling. After injection of the convulsants animals start to express status epilepticus (SE). Within 24 hours animals recover from SE and the seizure--free period, referred to as epileptogenesis (usually lasting up to one month) continues until the animal starts to express spontaneous seizures, equating to a diagnosis of epilepsy. Extensive neuronal injury in EN (and the adjacent piriform cortex) was already observed within few hours after SE [8, 33]. Moreover, the neurodegeneration continued during the course of the disease [7, 12, 32]. As a result, approximately one third of neurons were lost 10–12 months later [6, 7]. Interestingly, using Fos protein as a marker of neuronal activation, Willoughby et al. [45] demonstrated that during SE induced by kainic acid EN was not recruited before the first convulsive seizure appeared. A study by Ribak et al. [34] demonstrated that EN is also recruited in audiogenic seizure spread in genetically epilepsy-prone rats.

To summarise, EN is involved in the development and spread of seizures and is substantially affected by the epileptic process.

CONCLUDING REMARKS

The most striking features of EN connectivity are its heavy reciprocal projections with the piriform, perirhinal and entorhinal cortices as well as robust intrinsic connectivities through the whole rostrocaudal extent of this nucleus. The projections to a nuclear type of structure are lighter in density. When electrophysiological, pharmacological and anatomical studies are taken into account, it may be concluded that under the condition of altered excitability during the epileptic process EN may be involved in the propagation and generalisation of seizures originating in other brain regions.

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