Organization of projections from the mediodorsal nucleus of the thalamus to the basolateral complex of the amygdala in the rat

Eileen H.S. van Vulpen* and Ronald W.H. Verwer

Netherlands Institute for Brain Research, Amsterdam (The Netherlands)

(Accepted 5 July 1989)

Key words: Amygdala; Anterograde transport of Phaseolus vulgaris-leucoagglutinin; Basolateral complex; Mediodorsal thalamus

Mediodorsal thalamic (MD) projections to the basolateral amygdaloid complex of the rat were investigated with the anterograde neuronal tracer, Phaseolus vulgaris-leucoagglutinin. Iontophoretic injections were made in distinct subdivisions of the rostral and caudal part of the MD. Both the medial and lateral division of the MD showed a projection to the basolateral complex and there appears to be a topographical organization of the innervation in the rostrocaudal direction: the rostral and caudal part of the MD project to respectively the mid-rostrocaudal and rostral part of the basolateral complex.

Several studies have described the organization of the connections between the prefrontal cortex (PFC), the mediodorsal nucleus of the thalamus (MD) and the basolateral nucleus of the amygdala (BL). It has been proposed that these structures constitute a system called the basolateral limbic circuit (cf. refs. 2, 10, 18, 21, 22) which would be involved in learning and memory-related processes. It has been proposed that these 3 structures constitute a system called the basolateral limbic circuit (cf. refs. 2, 10, 18, 21, 22) which would be involved in learning and memory-related processes. The connections between PFC, MD and the BL have been reported to be reciprocal, though the reciprocity of the connection between the BL and the MD has been disputed.

Using radioactive amino acids, the projection was described from the medial segment of the MD to the BL in the rat and in a degeneration study in the monkey, a similar observation has been made. However, other investigators using retrogradely transported horseradish peroxidase (HRP) found no or very few cells labeled in the MD and it was suggested that this projection would be negligible. Methodological differences may have caused the discrepancy concerning the MD–BL projection in the rat. It is known that some cell systems are difficult to label with HRP and the possible existence of collaterals could also give rise to undetectable amounts of HRP in neurons (cf. ref. 5). On the other hand, it has been suggested that the anterograde tracer injections of Krettek and Price may have involved some nuclei adjacent to the MD. To resolve the controversy between anterograde and retrograde tracing techniques, we decided to use the anterograde neuronal tracer, Phaseolus vulgaris-leucoagglutinin, which is preferentially transported in the anterograde direction. This technique offers several advantages, as compared with other anterograde tracing methods. Small injections are possible and their size can be determined rather precisely. Another advantage is the good morphology of the labeled axons and axon terminals, which enables the delineation of the termination area precisely.

A total of 16 adult male Wistar rats (weighing 180–350 g) was used in this experiment. Under deep anesthesia with Hypnorm, Phaseolus vulgaris-leucoagglutinin (PHA-L; Vector Labs, U.S.A.; 20 µg/µl) dissolved in 0.15 M Tris-buffered saline, pH 7.6 (TBS), was injected iontophoretically in the mediodorsal nucleus of the thalamus (MD). Iontophoretic application was achieved through glass micropipettes (15–45 µm tip diameter) using a positively pulsed 6 µA DC current (5 s on, 5 s off) for 30 min. Six to 13 days after surgery, the rats were

* Present address: Neuroscience Unit, Montreal General Hospital, Montreal, HG3 1A4, Canada.
Correspondence: R.W.H. Verwer, Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands.
reanesthetized with sodium pentobarbital (0.1 mg/100 g b.wt.) and perfused intracardially with 50 ml 0.9% saline, immediately followed by 500 ml 2.5% glutaraldehyde, 1% paraformaldehyde in 0.05 M phosphate buffer, pH 7.6. The brains were removed from the skull and postfixed for 1 h in the same fixative. After postfixation, the brains were immersed in 0.05 M TBS and sectioned transversely at a thickness of 50 μm on a vibratome, and collected in TBS. The sections were incubated via the peroxidase–antiperoxidase (PAP) method according to Sternberger and all incubation steps were performed in TBS with the addition of Triton X-100 (0.5%). All antisera were raised in our own institute and specificity of staining was tested with brains that were not injected with PHA-L. The sections were mounted on glass slides and dried at room temperature. Selected sections were processed for Nissl-staining with thionine for delineation of the thalamic and amygdaloid nuclei. After staining, all sections were dehydrated in a graded series of alcohol and mounted with Entellan.

The basolateral amygdaloid complex can be divided in the lateral (LA) and basolateral nucleus (BL), of which the latter can be separated into an anterior (BLa), posterior (BLp) and ventral (BLv) part. The pattern of labeling in the basolateral complex of the amygdala, following injections of PHA-L in the distinct subdivisions of the mediodorsal nucleus of the thalamus, depend on the rostro-caudal location of the injection. Fig. 1 shows a representative example of injections placed in the medial part of the mediodorsal nucleus (MDm) at a rostral level. The spot was limited to the MD and no parts of the surrounding nuclei (notably the paraventricular nucleus at this level) were involved in the injection site (Fig. 1A). In the most rostral part of the basolateral complex a few fine fibers were present, which were usually straight with irregularly spaced varicosities. Sometimes branchlets with terminal-like varicosities were found. The fibers were predominantly found in the proximity of the medial and lateral border of the basolateral complex (Fig. 1E). At the same level many fine varicose axons were also observed in the substantia innominata and the medial amygdaloid nuclei (Fig. 1E). At a more caudal level, a large number of fine fibers appeared to run criss-cross along the medial and lateral border of the basolateral complex and also in the border zone between the lateral and the basolateral nucleus (Fig. 1F). Apart from these fine fibers, a small number of thick fibers with varicose branches running along the medial border were found. In the center of the BL very fine fibers, with a beaded appearance, were diffusely distributed. More caudally the fiber density became higher, except for the dorsolateral part of the lateral nucleus, where no fibers were present (Fig. 1G). At the same level, the ventral basolateral nucleus (BLv) contained many straight fibers running in the direction of the piriform cortex. In the ventral part of LA and the dorsal part of BLa many thick fibers with long branches and irregularly spaced varicosities run along the medial border (Fig. 1C). At the border of the ventral LA and the BLp, numerous fibers with many varicosities and short branches were present. At this level BLa showed a higher fiber density than BLp. The fibers in the BLa showed no specific orientation (Fig. 1D). At the most caudal level of the basolateral complex, the fiber density was quite low (Fig. 1H). Only a few fibers at the border of the ventromedial part of LA and the BLp with some varicosities were present.

By contrast, injections placed in the caudal part of the medial MD showed the highest density of fibers in the rostral part of the basolateral complex, where they were widely scattered. In the caudal part of the basolateral complex no fibers were seen. The central part of the MD was sometimes involved in both

Fig. 1. Injection of PHA-L in the MD with labeled fibers in the amygdala. A: photomicrograph of the spot involving the rostral part of the MDm. Nissl counterstaining. Scale bar = 2 mm. B: a drawing of the basolateral complex illustrating the locations of insets C and D. Nuclear borders were drawn according to Paxinos and Watson. The anterior-posterior level is comparable with Fig. 1G. C: photomicrograph showing the thick fibers with long varicose branches at the border between LA and BLa. Bar see Fig. 1D. D: photomicrograph showing the fiber density in BLa. Bar = 0.5 mm. E-H: rostral to caudal chartings of the anterogradely labeled fibers in the amygdala following the injection shown in A. The boundaries within the basolateral complex are according to Paxinos and Watson. AHi, amygdalohippocampal area; BLa, anterior basolateral nucleus; BLp, posterior basolateral nucleus; BLv, ventral basolateral nucleus; Ce, central amygdaloid nucleus; EnD, dorsal endopiriform nucleus; Me, medial amygdaloid nucleus; ot, optical tract; PRh, perirhinal cortex; st, stria terminalis.
rostral and caudal MDm injections. However, the innervation of the basolateral complex did not show obvious differences from the cases in which the injection was restricted to the MDm. Fig. 2 shows a representative example of injections placed in the lateral part of the mediodorsal nucleus (MDI) at a caudal level. There was minimal infiltration of the adjacent centrolateral and centromedial nuclei (Fig. 2A). At the most rostral part of the basolateral complex a large number of fibers, which were evenly distributed, was present (Fig. 2C) and most of them had a relatively thick appearance with many varicosities of various sizes. Occasional branches with the appearance of terminal boutons were seen. At a somewhat more caudal level, the basolateral complex showed the highest density of fibers (Fig. 2B,D), with the exception of the lateral border zone which was devoid of fibers. They were more or less

Fig. 2. Distribution of labeled fibers in the amygdala following injection of PHA-L in the MDI. A: photomicrograph of the injection site in the caudal part of MDI. Nissl counterstained. Bar = 2 mm. B: photomicrograph of the fibers in the basolateral complex. Shaded area indicates the environment. N.B. The lateral border of the complex contains no fibers. Bar = 0.5 mm. C-E: schematic drawings of the fiber distribution at different rostral to caudal levels. See Fig. 1 for symbols.
diffusely distributed, with a slight accumulation at the ventral border with the ventral endopiriform nucleus and at the medial border of the complex. More caudally, the innervation became less dense and more orientated along the boundaries of the complex, except for the dorsal border (Fig. 2E). A cluster of fibers was found running along the border between the lateral and the basolateral nucleus and also at the lateral border of the basolateral nucleus. The fibers, which were quite thin and straight, showed few varicosities. At the most caudal level of the basolateral complex no fibers were present. Thus, caudal MDI results can be compared with caudal MDm injections. There were no injections restricted to the rostral part of the MDI, therefore, no comparison can be made between rostral MDI and MDm injections.

As it appears from our results obtained with the PHA-L tracing method, the amygdala (and especially the basolateral nucleus) receives a projection from the mediodorsal nucleus of the thalamus in the rat. This projection, which reciprocates the projection from the BL to the MDm, is organized in a roughly topographic fashion. The rostral part of the MD has the greatest fiber density in the midrostrocaudal part of the basolateral complex and the caudal part of the MD projects primarily to the rostral part of the complex. This topographic organization shows a resemblance with the amygdalo-thalamic connection, because the rostral and caudal part of the MD projects preferentially to the rostral part of the BL. Thus, the results confirm and extend the observations made by Krettek and Price and Nauta, but are at variance with the results obtained with the retrograde HRP tracing. Nauta et al. noted that some projection systems are difficult to trace with HRP. This could be the case with the innervation of the MD from the BL, which could only be demonstrated with HRP after lesioning of the medial prefrontal cortex. However, with a fluorescent tracer, which is much more sensitive than HRP or WGA-HRP (cf. ref. 23), McDonald was able to show a clear projection from the BL to the MDm. A similar phenomenon could well be the case with the projection of the MD to the basolateral nucleus. It may be emphasized that other thalamic nuclei, which are noted for their projection to the basolateral nucleus (i.e. interanteromedial, parafascicular, paraventricular and parataenial nucleus), were not involved in our injections. Thus, the PHA-L technique proves the existence of a distinct projection from both MDm and MDI to the basolateral complex. The projection from the MD to the amygdala seems to be specifically oriented towards the basolateral complex, while some fibers are also present in the perirhinal cortex. This is in contrast with the reciprocal (i.e. amygdalo-thalamic) connection, where the fibers in the MDm arise from a population of neurons scattered throughout most of the amygdala without a specific topographic distribution. Some cells in the BL, which project to the MDm belong to a population of so-called class II cells. Viewing the organization of the cells in the basolateral complex, most of the spine-sparse class II cells were concentrated along Bla-BLp border, the border between the lateral nucleus and the caudal part of the basolateral nucleus, the medial and lateral border of the basolateral complex and at the ventral part of the BL. The present results indicate that the thalamic fibers within the basolateral complex are located preferentially along the borders and this could be an indication of mediodorsal thalamic fibers terminating at the class II population in the BL. Preliminary electron microscopical results showed that the labeled fibers also terminate in the basolateral nucleus. Most synapses were found on dendritic profiles, while numerous immunoreactive profiles were present in the vicinity of cell bodies. However, the triton treatment inherent in the immunocytochemical procedure resulted in an ultrastructure that did not permit any conclusions about the nature of the postsynaptic cells.

The prefrontal projection areas of the MDI and MDm, respectively the anterior cingulate cortex (cf. refs. 27, 29) and the prelimbic area (cf. refs. 28, 30), have been implicated in spatial learning and memory (see also Kolb). The above-mentioned prefrontal cortical areas also have reciprocal connections with the basolateral nucleus (cf. refs. 2, 12, 16, 25) and the innervation of the BL from the MD might represent an extra feedback in the basolateral limbic...
The authors are grateful to Drs. C.G. Van Eden and H.B.M. Uylings for critically reading the manuscript and to H. Stoffels for help in preparing the figures.
