European Journal of Morphology
1999, Vol. 37, Nos. 2, 3, pp. 185–189

0924-3860/99/3702/3-0185$15.00

A POLYSENSORY PATHWAY TO THE FOREBRAIN OF THE PIGEON: THE ASCENDING PROJECTIONS OF THE NUCLEUS DORSOLATERALIS POSTERIOR THALAMI (DLP)

O. Güntürkün and S. Kröner

Department of Biopsychology, Faculty of Psychology, Ruhr-Universität Bochum, Bochum, Germany

ABSTRACT

Visual evoked potentials (VEPs) in the associative neostriatum caudolaterale (NCL) have shorter latencies than those recorded in other visual forebrain areas. Therefore visual input into NCL probably stems from a subtelencephalic relay. Tracing experiments revealed a projection of the nucleus dorsolateralis posterior thalami (DLP) into those portions of NCL in which visual, auditory, and somatosensory afferents from intratelencephalic parasensory areas terminate. Since VEPs in NCL are abolished after DLP-lesions, this structure has to be the critical relay. However, DLP also projects to other associative forebrain areas and parts of the basal ganglia. Previous experiments had furthermore revealed that DLP-neurons integrate visual, auditory, and somatosensory inputs. Thus, the DLP-projection onto various associative forebrain areas represents a true polysensory thalamotelencephalic system.

KEYWORDS: Electrophysiology, pathway tracing, prefrontal cortex, caudolateral neostriatum.

INTRODUCTION

This study aims to clarify two questions regarding the organization of the avian neostriatum caudolaterale (NCL), an area thought to be equivalent to the prefrontal cortex (PFC). First, are visual evoked potentials (VEPs) that can be recorded in and around NCL due to a projection of the nucleus dorsolateralis posterior thalami (DLP) onto the caudal forebrain? Second, what are the detailed patterns of projections established by ascending DLP-fibers?

The thalamo- and the tectofugal pathways which terminate in the wulst and the ectostriatum, respectively, constitute the main ascending visual systems in birds. Latencies of VEPs are about 22 ms for the Wulst and more than 50 ms for the ectostriatum (Parker & Delius, 1972). Since Güntürkün (1984) could record VEPs with latencies of 15 ms in the caudal neostriatum, and since these VEPs were unaffected by Wulst lesions, it was concluded that an independent 'third primary visual system' had to exist which terminated in the caudal forebrain. However, the thalamic relay of this pathway remained unknown.

Due to its dense dopaminergic innervation (Divac & Mogensen, 1985; Waldmann & Güntürkün), and its multimodal organization (Metzger et al., 1998; Kröner & Güntürkün, in press), the NCL was suggested to be comparable to the mammalian prefrontal cortex. Indeed, behavioural studies could demonstrate NCL-lesions to cause deficits in working memory and behavioural flexibility tasks (Gagliardo et al., 1996; Hartmann & Güntürkün, 1998), similar to the situation in PFC. The main thalamic afferents of NCL are the DLP and cells in the adjacent ventrointermediate

Address for correspondence: Dr. Onur Güntürkün, Biopsychologie, Fakultät für Psychologie, Ruhr-Universität Bochum, D-44780 Bochum, Germany. Fax: +49-234-7094377; e-mail: onur.guentuerkuen@ruhr-uni-bochum.de

area of the posterior nuclei (VIP) **(**Kröner & Güntürkün, in press). The DLP has been shown to constitute a polysensory entity in which visual, somatosensory, and auditory afferents converge on single neurons (Korzeniewska & Güntürkün, 1990) and which plays a role in working memory tasks (Güntürkün, 1997). Since the DLP is known to project to NCL (Waldmann & Güntürkün, 1993), the aim of the present study was to clarify if DLP is the missing thalamic link of the 'third primary visual system' and whether all parts of NCL receive input from DLP

MATERIALS AND METHODS

Due to the multitude of approaches methods are described only briefly and we rather refer to studies, in which these are described in more detail.

Anterograde tracing

A 2.5% solution of *Phaseolus vulgaris leucoagglutinin* (PHA-L) diluted in 0.1 M phosphate buffer (PB, pH 7.2) was injected in two animals over 30 min into the DLP using pulsed positive DC current of 4.5 μA. After 5 days animals were anesthetized and perfused first with 0.9% NaCl (40°C) followed by 4% paraformaldehyde (PFA) in sodium acetate buffer (pH 6.5) and finally by 4% PFA in sodium borate buffer (pH 9.5). Brains were postfixed in 4% PFA in acetate buffer (pH 6.5) for 1 hour and stored in 30% sucrose in PB at 4°C. Then, 25 m frozen frontal brain sections were cut and processed for ABC immunocytochemistry. For details of the staining procedure see Gerfen and Sawchenko (1983).

Retrograde tracing

In 12 pigeons, small amounts of Fast Blue (FB) or rhodamine isothiocyanate (RITC), each dissolved at 2% in 1% DMSO in distilled water were injected stereotaxically into NCL or the terminal field of DLP within the neostriatum intermedium medialis (NIM). An additional 16 animals received pressure injections of cholera toxin b (CTb) into the same areas within NCL or NIM. After 2–3 days animals were perfused with 0.9% NaCl followed by 4% PFA in PB (pH 7.2, 4°C). Brains were postfixed between 1 and 24 h (4°C) in the fixative with 30% sucrose added, and cut frontally in frozen sections of 40 m. Fluorescent tracers were visualized using a fluorescence microscope. For details see Korzeniewska and Güntürkün (1990). Immunohistochemistry for CTb followed the procedure described by Shimizu et al. (1995). In brief, slices were incubated overnight (4°C) in a primary antibody against CTb (1:20.000, containing 0.3% Triton X), followed by a goat biotinylated antibody (1:500 in 0.3% Triton PB; both Jackson) and finally the avidin-biotin complex (1:100; Vector). Staining was achieved by the 3,3′-diaminobenzidine-(DAB) technique.

Electrophysiology

Eight adult pigeons were anesthetized with equithesin and four 0.12 mm thick insulated stainless steel wires were implanted under stereotaxic guidance into the DLP-termination zone of the NCL of each hemisphere. Electrodes tips were staggered in 1 mm steps. An uninsulated loop of wire placed under the scalp served as reference. Free ends of electrodes terminated in a minuature connector glued onto the skull. Recordings were performed in awake animals. A red light diode (665 nm, half-bandwidth: 30 nm, intensity: 0.8 mcd, stimulus duration: 5 ms, interstimulus interval: 2.3 s) was placed 5 mm from the eye contralateral to recording. Potentials from electrodepairs were bandpass filtered between 7 and 120 Hz with a notch filter at 50 Hz, and were averaged over 32 trials. At the end of recordings small radiofrequency lesions were made at electrode tips to histologically verify their locations. In three pigeons a lesion electrode was placed in a second surgery into the DLP ipsilateral to the recording site and thermal coagulations (20 mA, 8 s) were made. NCL-recordings were made a few days before and one week after lesions. For further details see Güntürkün (1984).

RESULTS

Visual evoked potentials (VEPs) with first inflection latencies 16 to 22 ms were recorded from NCL. The area examined reached from A 4.5 to A 6.75 and from L 2.00 to L 8.00. Structure and latencies of VEPs were in close accordance with Güntürkün (1984). To test whether the initial VEP components derived from the DLP-projection onto NCL, the DLP was thermally lesioned (Fig. 1A). Postoperative recordings revealed a complete absence of VEPs (Fig. 1A).

PHA-L injections were centered on DLP but also slightly encroached upon surrounding VIP, nucleus dorsointermedius posterior thalami (DIP) and nucle-

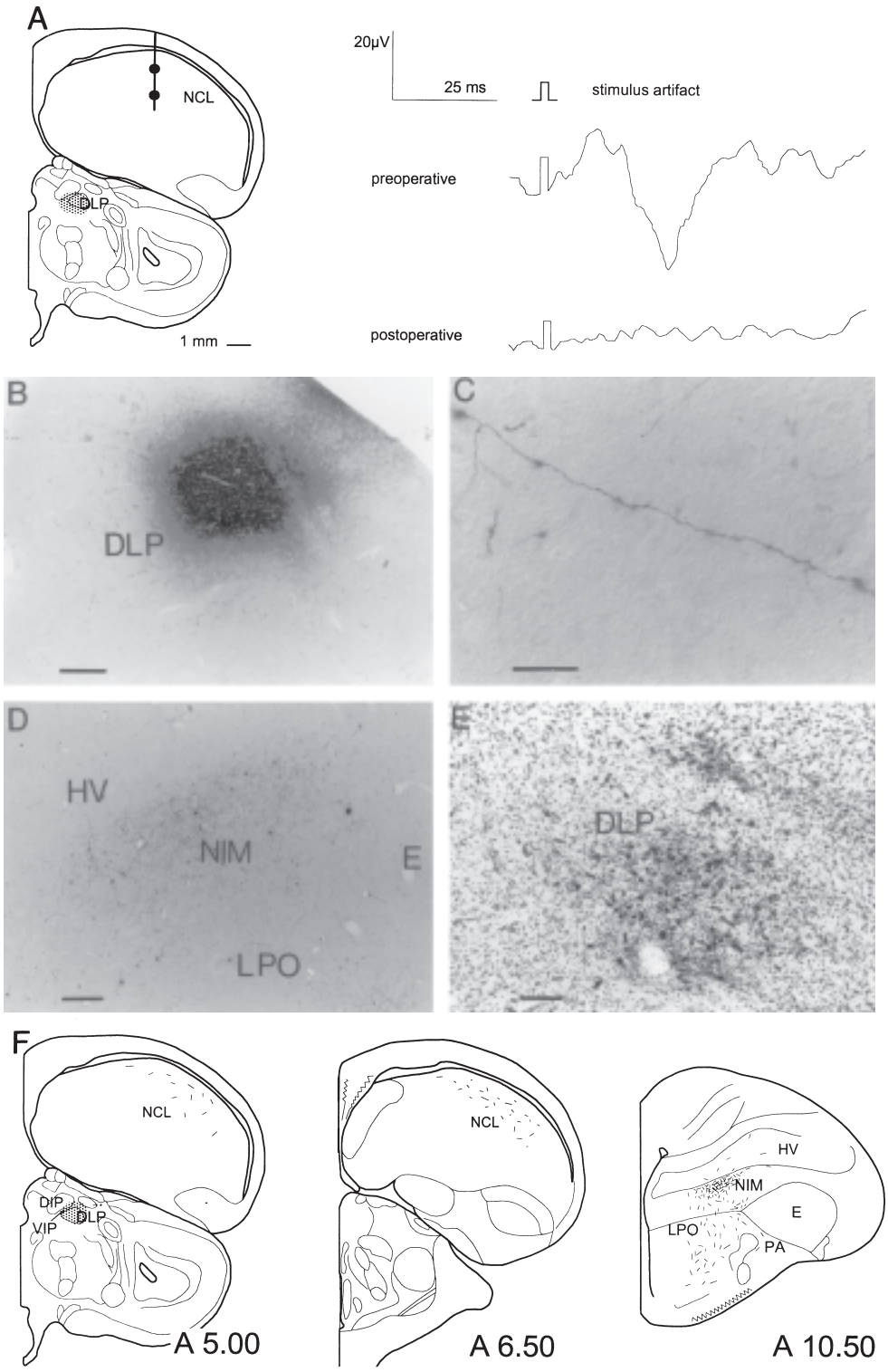

Fig. 1. (A) Diagrammatical summary of the electrophysiological experiments. The left part of the diagramm shows the location of the recording sites within NCL and the extent of the DLP lesion. The right part shows examples of VEPs before and after DLP lesion. (B) Injection site of PHA-L in the dorsal thalamus. (C) Anterogradely labeled fibres in the NCL following the PHA-L injection shown in B. (D) Terminal field of DLP afferents in NIM. (E) Retrogradely labeled cells in DLP after a CTb injection into the neostriatal area shown in D. (F) Diagrammatic representation of the DLP terminal fields in NIM and NCL. Scale bars are 500 µm (B,D), 100 m (C), and 250 µm (D).Abbreviations: E, ectostriatum; DIP, nucleus dorsointermedius posterior thalami; DLP, nucleus dorsolateralis posterior thalami; HV, hyperstriatum ventrale; LPO, lobus parolfactorius; NCL, neostriatum caudolaterale; NIM, neostriatum intermedium medialis; PA, paleostriatum augmentatum; VIP, ventrointermediate area of the posterior nuclei.

us dorsomedialis anterior thalami (DMA) (Fig. 1B). Labeled axons were observed to proceed rostrally to the fasciculus prosencephali lateralis (FPL). On their ascending route, fibers made dense terminal endings on neurons of the nucleus dorsointermedius ventralis anterior (DIVA) and the nucleus reticularis superior dorsalis. Many labeled axons could also be seen in the "somatic" parts of the basal ganglia, namely the lateral lobus parolfactorius (LPO) and the medial paleostriatum augmentatum (PA). Some fibres were seen dorsal to the lamina hyperstriatica (LH) in the ventral hyperstriatum (HV), the largest terminal field however extended underneath the LH just medially and dorsally of the ectostriatum in the NIM. Some fibres coursed frontally and terminated on neurons of the lamina frontalis superior (LFS). Others proceeded caudally to NCL, where fibers were seen to be loosely distributed and making terminal endings (Figs. 1C, F). These axons mainly concentrated in the caudodorsal aspect of NCL following broadly the previously described semilunar outline of the NCL (Waldmann & Güntürkün, 1993). The majority of fibres terminated in an area reaching from A 4.75 to A 7.25 and L 4.5 to L 8.5 within NCL (Figs. 1D, F). Only very few fibres extended into far lateral and ventral parts of NCL. Injections of retrograde tracers into NCL caudal to A 5.00 revealed few labeled cells in DLP. Injections placed further rostrally labeled neurons in VIP and throughout the complete rostrocaudal extent of DLP. Injections of retrograde tracers into the dense terminal field of the DLP within NIM resulted in a very large number of cells within DLP (Fig 1E). Injections into LFS revealed a small number of labeled cells in dorsal DLP.

DISCUSSION

This study clearly reveals that the DLP projects to a number of forebrain somatic and association areas. It could additionally be shown that the thalamic component of the 'third visual system' proposed by Güntürkün (1984) is the DLP, projecting to large parts of NCL.

The prime argument to propose an independent third visual system to the avian forebrain had been the latencies of VEPs which were shorter in the caudal forebrain then in the Wulst and the ectostriatum (Güntürkün, 1984). DLP integrates besides auditory, and somatosensory afferents also a massive visual input from the tectum. Since DLP projects onto NCL, the VEPs of the caudal forebrain are likely due to ascending DLP-projections. This assumption is clearly supported by the absence of VEPs in NCL after DLP-lesions. In addition to afferents from DLP, NCL integrates further visual input from secondary visual forebrain structures like caudal hyperstriatum accessorium (HA) and ectostriatal belt (Eb) (Metzger et al., 1998; Kröner & Güntürkün, in press). It is possible that these afferents contribute to the late VEP components. However, DLP-lesions had completely diminished VEPs, including their late components. This might be due to the cellular sensory properties of the structures involved. Korzeniewska and Güntürkün (1990) demonstrated that DLP-neurons had broad receptive fields and responded to simple stimulus properties. Given the possible complexity of reponse properties in secondary visual association areas of the forebrain, the simple diode-flash induced VEPs are likely to activate DLP-, but not HA- and Eb-neurons. The dorsal part of NCL which receives the densest DLP innervation has also been shown to receive auditory input via field L, afferents from visual and somatosensory parts of HA, as well as a projection from cells in the terminal field of the DLP within NIM (Kröner & Güntürkün, in press). It thus seems that the dorsal NCL is reached by intratelencephalic input from those modalities that are also transported by DLP afferents, and, in addition, those parts of NCL receive an indirect input from DLP via NIM. In summary, the DLP supplies the NCL with both a fast and a slow copy of incoming sensory information from all main modalities, which within NCL can be further compared with other input from the same modalities that originates in higher sensory areas of the telencephalon.

ACKNOWLEDGEMENTS

Thanks to our old master Juan Delius for his continuing support, his never ending enthusiasm, and his bright ideas. Supported by the Deutsche Forschungsgemeinschaft through its Sonderforschungsbereich 509 NEUROVISION.

REFERENCES

Divac I, Mogensen J (1985): The prefrontal "cortex" in the pigeon – catecholamine histofluorescence. *Neuroscience 15*: 677–682.

Gagliardo A, Bonadonna F, Divac I (1996): Behavioural effects of ablations of the presumed "prefrontal cortex" or the corticoid in pigeons. *Behav Brain Res 78*: 155–162.

Gerfen CR, Sawchenko PE (1983): An anterograde neuroanatomical tracing method that shows the detailed morphology of neurons, their axons and terminals: Immunohistochemical localization of an axonally transported plant lectin, *Phaseolus vulgaris leucoagglutinin*. *Brain Res 290*: 219–238.

Güntürkün O (1984): Evidence for a third primary visual area in the telencephalon of the pigeon. *Brain Res 294*: 247–254.

Güntürkün O (1997): Cognitive impairments after lesions of the neostriatum caudolaterale and its thalamic afferent in pigeons: Functional equivalencies to the mammalian prefrontal system? *J Brain Res 1*: 133–144.

Hartmann B, Güntürkün O (1998): Selective deficits in reversal learning after neostriatum caudolaterale lesions in pigeons – possible behavioral equivalencies to the mammalian prefrontal system. *Behav Res 96:* 125–133.

Korzeniewska E, Güntürkün O (1990): Sensory properties and afferents of the n. dorsolateralis posterior thalami (DLP) of the pigeon. *J Comp Neurol 292*: 457–479.

Kröner S, Güntürkün O (1999): Afferent and efferent connections of the caudolateral neostriatum in the pigeon (*Columba livia*): A retro- and anterograde pathway tracing study. *J Comp Neurol,* in press.

Metzger M, Jiang S, Braun K (1998): Organization of the dorsocaudal neostriatal complex: a retrograde and anterograde tracing study in the domestic chick with special emphasis on pathways relevant to imprinting. *J Comp Neurol 395*: 380–404.

Parker DM, Delius JD (1972): Visual evoked potentials in the forebrain of the pigeon, *Exp Brain Res 14*: 198–209.

Shimizu T, Cox K, Karten HJ (1995): Intratelencephalic projections of the visual wulst in pigeons (*Columba livia*). *J Comp Neurol 359*: 551–572.

Waldmann C, Güntürkün O (1993): The dopaminergic innervation of the pigeon caudolateral forebrain: Immunocytochemical evidence for a "prefrontal cortex" in birds? *Brain Res 600*: 225–234.

Accepted: September 16, 1998