

Projection of the Lateral Geniculate Nucleus onto Cortical Area V2 in the Macaque Monkey*

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Summary. Recent publications have demonstrated a projection of the lateral geniculate nucleus (LGN) onto extrastriate cortical regions in the old world monkey but have failed to identify a projection from this nucleus to V2, the area adjacent to the striate cortex. In this report we show that such a projection exists, as demonstrated by the retrograde transport of the fluorescent labels fast blue (FB) and diaminidino yellow (DY). Neurons labelled after V2 injections are more scattered in the LGN than the cells backfilled by the V1 injections and mostly belong to the interlaminar zones and the S layers, regions which are largely devoid of neurons labelled by the V1 injections.

Key words: Monkey – LGN – V2 – Interlaminar zones – Double labelling

Introduction

Until recently it has been thought that, in old world monkeys, the lateral geniculate nucleus (LGN) projects only to the striate cortex via relay cells in the laminae. The use of more sensitive anatomical tracing techniques has revealed the presence of a projection from both the laminae and the interlaminar zones of the LGN to extrastriate areas (Yukie and Iwai 1981; Benevento and Yoshida 1981; Fries 1981). These reports were based on horseradish peroxidase (HRP) injections into the posterior bank of the superior temporal sulcus, the prelunate gyrus and the anterior bank of the lunate sulcus. Comparison of the sites of HRP injections with visual maps in prestriate

cortex indicates that the injected regions probably correspond to V4 and Zeki's motion area (Zeki 1974; Van Essen and Zeki 1978).

Investigations of the thalamic afferents to the posterior bank of the lunate sulcus has failed to reveal projections from the LGN (Benevento and Yoshida 1981; Benevento and Standage 1982). This cortical region is mostly occupied by V2 which is the visual area immediately adjacent to the striate cortex (Van Essen and Zeki 1978; Gattass et al. 1981). The reported lack of LGN projection to the visual area adjacent to area 17 in the monkey is surprising since it was this cortical region which was innervated in all three other species where a LGN projection outside area 17 has been found (rat: Coleman and Clerici 1980; sheep: Karamanlidis et al. 1979; cat: Garey and Powell 1967; Glickstein et al. 1967). In this paper, we report that the LGN does project to V2 in the old world monkey. These results, which are based on the retrograde transport of fluorescent tracers, also demonstrate that this projection arises mainly from the interlaminar zones of the LGN.

Material and Methods

The retrograde tracers used in this study were the fluorescent dyes diaminidino yellow (DY) and fast blue (FB). The properties of these compounds are described in detail in a recent publication by Kuypers's group (Keizer et al. 1983). Injections were made with Hamilton syringes in adult cynomolgus monkeys (*Macaca Irus*) anesthetized with ketamine hydrochloride (15 mg/kg i.m.) followed by pentobarbitone sodium (5–15 mg/kg i.v.). Each animal was given a single injection of one fluorescent dye in V1 and an injection of the other dye in V2. The injections were arranged in such a way that part of both injections would concern retinotopically corresponding regions in both areas. The injections in V1 were made on the operculum at some distance (5–10 mm) and parallel to the V1–V2 border and covered a long distance (5–10 mm) at a shallow angle to the cortical surface. The V2 injections were made perpendicular to the horizontal Horsley-

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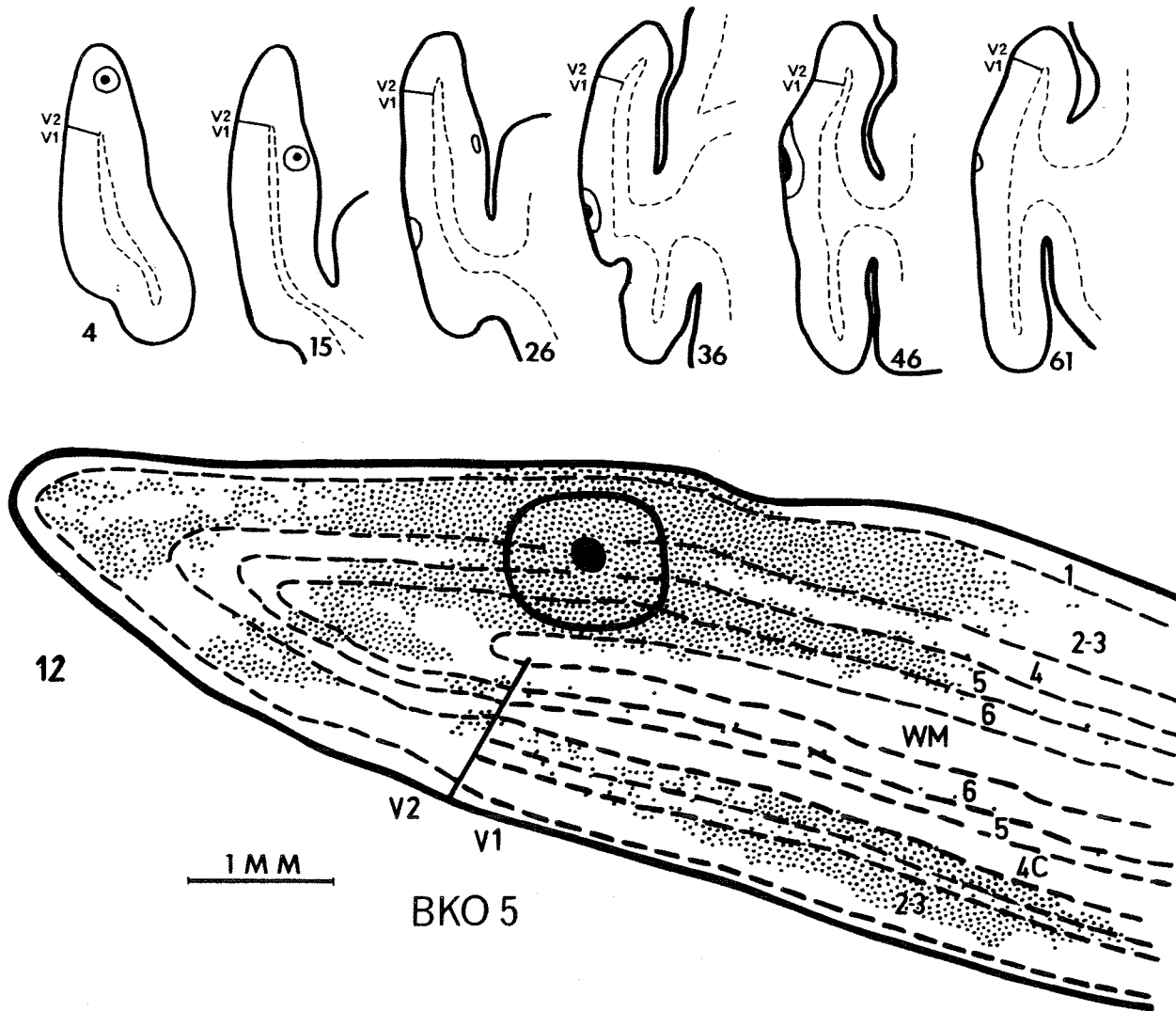


Fig. 1. Injection sites in V1 and V2: the upper series of horizontal sections shows the extent of the DY injection in V2 and of the FB injection in V1. The *black area* represents the needle track and the region enclosed in the *heavy line* surrounding the track corresponds to the region of dense coloring where pick-up occurs. Higher section numbers correspond to lower levels in the brain. The expanded view of section 12 shows the DY injection with the needle track (*dark disk*) surrounded by a region of dense yellow color (*circled by a heavy line*) and by neurons labelled by the DY dye (*small dots*). Note that the population of labelled neurons in V2 extends further laterally in laminae 2-3 and 5-6 than in lamina 4. This presumably results from the small lateral spread of axons of lamina 4 neurons and indicates that the region where the dye is picked up corresponds to the zone of dense yellow color *circled by the heavy line* (see text)

Clarke plane and spanned 4-6 mm in the posterior bank of the lunate sulcus (Fig. 1). Injected volumes were of the order of 0.5-1 μ l (0.1 μ l per mm) and the concentrations were 2% for the DY and 3% for the FB.

After a survival period of 7-11 days, the animals were perfused transcardially with 1 l of 2.7% saline, followed by 3 l of 30% formalin in 0.1 M phosphate buffer, 1 l of 8% sucrose, 1 l of 20% sucrose and 1 l of 30% sucrose. The brain was immediately removed, blocked and cut on a freezing microtome at 40 μ , every third section being kept. The sections were mounted from phosphate buffer or saline onto gelatinized slides and observed under deep blue and UV light using a Leitz microscope with a D filter set (355-425 nm) and oil immersion objectives (X10, X25,

X50, X100). Under this illumination, the FB-labelled neurons exhibit a blue coloration in their cytoplasm and the DY-labelled neurons show a yellow nucleus (Keizer et al. 1983). The sections were traced and the position of labelled neurons plotted by means of an X-Y plotting table electronically coupled to the microscope stage. In the case of the LGN the presence in neurons of lipofuscin, which fluoresces yellow-orange in UV illumination, was used to draw the outlines of the structure with its laminae at the time of observation. For other structures where precision was less critical, the sections were counterstained with cresyl violet after observation. The outlines of the nuclei were then reconstructed and the match made with the drawn sections using landmarks such as the pial border and the blood vessels.

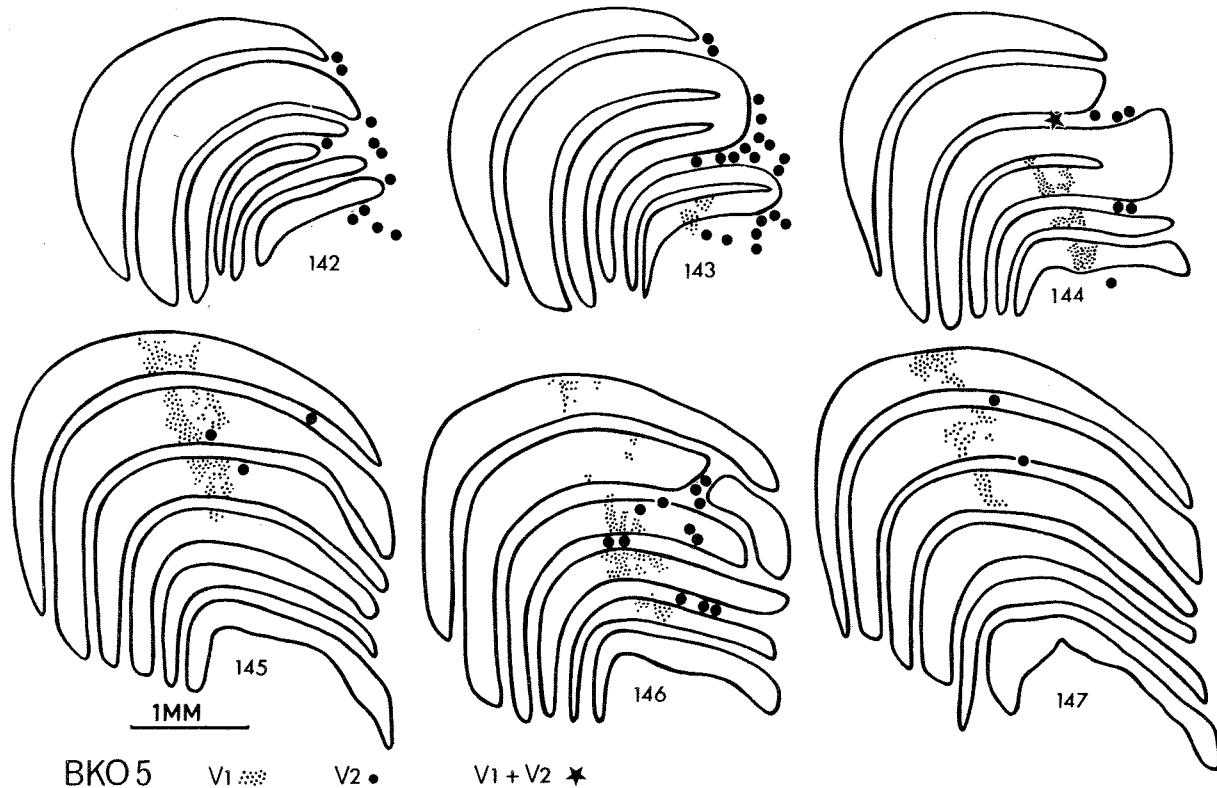


Fig. 2. Labeled neurons in the LGN after the injections shown in Fig. 1. Note that V1 labelled neurons are concentrated in a narrow column, whereas the V2 labelled cells are more scattered and more concentrated around the laminae, in the interlaminar zone and the S layers. One double labelled neuron is indicated by a *star*

Results

The results are based on five animals, four were injected in V1 and V2 and one in V2 and the prelunate gyrus. Following injections of V1 and V2, labelled cells containing either dye were found in the LGN. Representative injection sites are shown in Fig. 1 and the pattern of labelled LGN cells in Fig. 2. Labeled cells projecting to striate cortex formed a narrow column in the LGN. The majority of cells were in the laminae, with a few in the interlaminar zones. The neurons labelled by the dye injected in V2 showed a different location within the LGN. First, the cells labelled with the V2 dye were more scattered in the LGN than those labelled with the dye injected in V1 (Fig. 2). Secondly, they were concentrated in the interlaminar zones and the S layers (Kaas et al. 1978). Table 1 presents the distribution of labelled neurons in the laminae and interlaminar zones. Between 46 and 91% of the cells projecting to V2 were found in the interlaminar zones and the S layers. In the region where the populations of V1 and V2 backfilled neurons overlapped, only a few double labelled cells were found which were all restricted to

the interlaminar zones (Table 1), indicating that in the monkey few LGN neurons send branching axons to V1 and V2.

The injection in Fig. 1 is typical in that the needle track of the V2 injection came close to the V1-V2 border. For this reason, it was crucial to estimate the extent of the pick up area of the dyes used. This estimation was done in a cat where we made two injections, one of fast blue, one of diamidino yellow, separated by 3.8 mm. The resulting blue and yellow columns in the LGN were clearly separated and a simple computation demonstrates that the pick-up zone of either dye extends laterally by no more than 0.5 mm on either side of the needle track and corresponds approximately to the zone of dense color surrounding the center of the injection (Bullier, Kennedy and Salinger, to be published). Confirmatory evidence comes from examination of the lower part of Fig. 1. The pattern of labelled neurons surrounding the injection track has a characteristic H-shape pattern which was found in all the injected animals. The arms of the H are constituted by the populations of neurons in layer 2 and 3 and in 5 and 6 extending up to 3 mm from the injection track. The

Table 1. LGN neurons labelled after injection in V1 and V2

Case	BKO1	BKO2	BKO4	BKO5	BKO7
Injection sites	DY in V1 FB in V2	FB in V1 DY in V2	FB in V1 DY in V2	FB in V1 DY in V2	FB in prelunate gyrus DY in V2
Number of 40 μ sections sampled	7	6	6	7	5
Number of neurons in interlaminar zones and S layers projecting to V2	28 (76%)	19 (46%)	48 (65%)	52 (91%)	25 (68%)
Number of neurons in the main laminae projecting to V2	8 (22%)	22 (54%)	22 (30%)	4 (7%)	12 (32%)
Number of double labelled neurons after V1 and V2 injections	1 (2%)	not counted	4 (5%)	1 (2%)	not applicable

short bar of the H is made up of the population of labelled neurons in lamina 4 which do not extend far laterally. A plausible explanation of this pattern around the injection site rests on the known interconnectivity within the cortex. Axons of layer 4 neurons do not extend far laterally whereas neurons in infra- and supra-granular layers have axons which send recurrent collaterals several millimeters from the cell body (Lund et al. 1981). The limited extent of the population of labelled cells in layer 4 confirms, therefore, that the pick-up area is small and corresponds to the zone of dense coloring mentioned above. As this region did not invade V1 or the white matter, one can conclude that the V2 injections were restricted to this area.

In one animal (BKO7) we injected DY in V2 and FB in the prelunate gyrus. The pattern of labelling was similar for both dyes in that neurons of either color were relatively scattered in the LGN and belonged mostly to the interlaminar zones and S layers. In the five sections where we counted neurons, we found 57 neurons labelled by the dye injected in the prelunate gyrus. In this population, 59% of the neurons belonged to the interlaminar zones, a figure comparable to the proportion of such neurons labelled after V2 injections. In addition, we observed nine double-labelled neurons of various size and shape, which suggests that the proportion of LGN neurons which send branching axons to V2 and the prelunate gyrus may be higher than in the projection to V1 and V2.

Discussion

The validity of the results rests on the lack of flooding of the dye injected in V2 into other areas. The small

size of the pick-up zone and its distance from the V1-V2 border and the white matter indicated that the dye could not penetrate into V1. This is confirmed by the different distribution of the LGN neurons labelled by the V1 and V2 injections (Fig. 2). If the dye injected in V2 had spilled over into V1, the labelled neurons would have mostly occupied the laminae and have been restricted to a small zone in the LGN. The possibility that the V2 injection spilled over in the cortical regions anterior to V2 is negligible as the needle track did not approach the fundus of the lunate sulcus where other extrastriate visual areas are found (Van Essen and Zeki 1978). It is also unlikely that the dye injected in V2 spread across the lunate sulcus to invade the anterior bank as there were few labelled cells in the supragranular layers of this region of cortex. Had the dye crossed the lunate sulcus, the anterior bank would be expected to contain large numbers of locally labelled cells in the upper layers.

One could argue that labelled neurons in the LGN result from transynaptic retrograde transport of the dye injected in V2. This possibility appears unlikely as, for both dyes used, neurons were as strongly labelled after V2 injections as after V1 or prelunate gyrus injections. We specifically controlled for the possibility of transynaptic transport by examining the retinae of one animal with a strong injection of FB in V1. Despite heavy anterograde and retrograde labelling in the LGN, no labelled neuron was found in the retinae.

The failure of previous workers to demonstrate a projection from the LGN to V2 in the monkey (Benevento and Yoshida 1981; Benevento and Standa 1982) may result from the lower sensitivity of

the HRP method compared with the retrograde transport of fluorescent dyes. This is reflected in the presence in our study of neurons in infragranular layers of V1 labelled after injections in V2. It is unlikely that labelling in these neurons resulted from pick-up of the dye in the white matter as the marked cells were localized at the 5-6 border (Fig. 2). This hitherto unknown projection (Rockland and Pandya 1979; Lund et al. 1981) is also found between V1 and V2 in the cat (Bullier, Kennedy and Salinger, unpublished results) and probably corresponds to the presence of axon collaterals going to V2 in layer 5 neurons intracellularly injected with horseradish peroxidase (Martin and Whitteridge, personal communication).

In conclusion, our results show that the LGN of old world monkeys projects to V2 as well as to other prestriate areas. This projection, however, does not appear to be homologous to the projection from the LGN to V2 in the cat. In this species, the LGN neurons projecting to V2 belong to the main laminae which relay information coming directly from the retina to lamina 4 of V1 and V2. In the monkey, the connection between the LGN and V2 arises mostly from the interlaminar zones and the S layers which are known to receive afferents from the superior colliculus (Harting et al. 1980; Benevento and Yoshida 1981). These regions, in turn, convey the visual information coming from the superior colliculus to extrastriate areas and to supragranular layers of the striate cortex (Weber et al. 1983; Carey et al. 1979). As suggested by Benevento and Yoshida (1981) and Weber et al. (1983), it would thus appear that the LGN projection to V2 in old world monkey is part of a retino-colliculo-thalamic pathway rather than a retino-thalamic pathway to the visual cortex as is the case in the cat visual system.

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