SPECIALIZATION AUDITORY PATHWAYS IN THE MOLE

43.80.Lb Sound reception by animals: anatomy, physiology, auditory capacities, processing

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ABSTRACT:
To get morphological substrates for sensory specialization in the subterranean mammals, we investigate both auditory and visual pathways in the mole. The inferior colliculus (IC), an auditory relay, projects not only to medial geniculate, the major gateway to the auditory cortex, but also to the lateral geniculate (LG), the major gateway to the visual cortex. Further evidence is that the LG does not send many fibers to the cortex in the mole. Instead, the auditory inputs to the LG are likely to be conveyed to the suprachiasmatic hypothalamic nucleus (SCN), which plays a role in photoperiodic functions in common mammals. Auditory inputs to the SCN may subserve periodic reproductive behaviors in the exclusively separated territorial domains of subterranean mammals.

INTRODUCTION

The insectivorous mole, *Talpa/Mogera* (Nowak, and Paradiso,1983), an underground dweller, is one

of the most primitive and ancestral mammals, least modified since the Paleocene period, where radiative evolution of mammals has begun (Simpson, 1945). The mole has highly regressive eyes, and is almost blind (Lund and Lund, 1965; Quilium, 1966), as is the mole rat *Spalax*, a rodent counterpart of the mole (Necker et al., 1992). Due to curiosity concerning the evolutionary selection pressure favoring reduction of the eye and the consequent regression of the central visual system, the brain of these burrowing mammals has been an attractive research subject.

In the subcortical visual pathways of these animals, the lateral geniculate body (LG) in the thalamus and the superior colliculus (SC) in the midbrain are highly regressed, whereas the suprachiasmatic hypothalamic nucleus (SCN) is well developed (Kudo et al., 1991; Kudo et al., 1988; Cooper et al., 1993a,b). It is assumed that the organization of each of the visual pathways in these animals may have been modified during phylogeny , reflecting its functional significance in the subterranian environment (Aboitiz, 1993). Indeed, 'intermodal' modifications may have occurred in the organization of sensory pathways of some blind vertebrates. In *Spalax*, the LG, a major thalamic relay nucleus in the central visual pathway for form vision, receives auditory inputs from the inferior colliculus (IC), the major relay in the central auditory pathway (Doron and Wollberg, 1994). In the blind cave fish (*Astyanax hubbsi*) an invasion of somatosensory fibers into the visual system occurs in the superficial layers of the tectum, which are thought to be purely visual (Voneida and Fish, 1984). Thus, investigation of the visual system in subterranean animals with reduced eyes is expected to provide,, not only an understanding of the impact of peripheral reduction on the organization of central nervous system but also an opportunity to determine whether such cross-modal modification occurs in the brain organization of animals adapting radically to the subterranean environment.

In the present report, we examined both visual and auditory pathways in the insectivorous mole *Mogera*, which is equipped with characteristically organized peripheral auditory apparatus (Henson, 1988; Coles et al., 1982), as well as with highly reduced eyes. To trace these pathways, we used various tract-tracing markers, which were anterogradely, retrogradely or transneuronally transported by axonal flow.

MATERIALS AND METHODS

For all surgical procedures, animals were anesthetized with a mixture containing sodium pentobarbital (16.2mg/kg, intraperitrium administration) and chloral hydrate (50mg/kg, intraperiatrium administration). In five animals, o.2% cholera toxin subunit B conjugated to horseradish peroxidase (CT-HRP, List Biological Lab.) was injected into one eyeball. In 15 animals, 4% wheatgerm agglutinated horseradish peroxidase (WGA-HRP), Toyobo) was injected unilaterally into the IC (12 animals) or into the SC (three animals). In nine animals, 10% Fluoro-ruby (FR, dextrantetramethylrhodamine, Molecular Probes) was injected into the occipital (four), the parietal (three) or the temporal cortex (two). In three animals, 1% tetanus toxin C fragment (TTC, Boehringer Mannheim) was injected into one eyeball. All the injections were made through a glass micropipette (8-20 µm in tip diameter) by air pressure.

Following a survival period of 20-48 hours, the animals were deeply anesthetized and perfused transcardially with 300ml 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.3), followed by 100 ml of the same buffer containing 5% sucrose. The brains were removed immediately, saturated with 20% sucrose in the same buffer at 4 C, and cut at 40µm in the coronal plane on a freezing microtome.

For horseradish peroxidase histochemistry (CT-HRP and WGA-HRP), the brain tissue was processed and treated by standard methods (Mesulam, 1978). For FR, the sections were mounted on glass slides serially, left at room temperature overnight, and observed under a fluorescent microscope (Kuypers and Huisman, 1984). For the immunohistochemical detection of transported TTC, we followed the method of Mannings *et al*. (1990). We used the anti-TTC monoclonal antibody, which has been commercially prepared (Boehringer Mannheim). The other series of alternate sections mounted on glass slides were Nissl-stained with Cresyl violet for cytoarchitectnic verification in all cases. Further series of sections were incubated for acetylcholineesterase (AchE) histochemistry in some cases.

RESULTS

In five animals, an anterograde tracer, CT-HRP, was injected into the eyeball, the pattern of distribution of retinal fibers labeled anterogradely with CT-HRP was essentially the sane as that observed previously in moles which were injected intraocularly with the anterograde tracer WGA-HRP (Kudo et al., 1991; Kudo et al., 1988). Terminal labeling was observed in the well developed SCN of the hypothalamus most obviously. This is a great discovery that such animals with such poor vision still need to adjust their biological clock, i.e. SCN. Other central visual centers were generally reduced; the LG, the lateroposterior nucleus in the thalamus and part of pretectum received a few retinal fibers. The superficial visual layer of the SC was completely lacking. The terminal labeling in the well developed SCN was seen bilaterally with a slight ipsilateral predominance; almost other visual regions were observed contralaterally. No terminal labeling was observed in the SC. The LG of the mole is contiguous to the lateroposterior and laterodorsal thalamic nuclei along the dorsolateral border of the thalamus. Terminal labeling was observed throughout the entire LG.

In Nissl-stained preparations, the LG was cytoarchitectonically divided into a dorsal and a ventral part; darkly stained, uniform-sized cells were densely distributed in the dorsal division, while less darkly stained cells were loosely arranged in the ventral division. These dorsal and ventral divisions were considered to correspond to the dorsal nucleus (LGd) and ventral nucleus (LGv), respectively. AchE histochemistry also revealed a difference between the LGd and the LGv; along with the thalamic reticular nucleus, the LGv was stained more densely than the LGd. The fact that the LGv and the thalamic reticular nucleus both give rise to the descending projections to the SC and the pretectum may also justify the present manner of subdivision of the LG into the LGd and LGv.

In five of 12 animals which were injected with an anterograde tracer, WGA-HRP, unilaterally into the IC, the sites of the injection were strictly confined to the IC. In these five animals, no WGA-HRP diffusion into

the SC was detected; resultant terminal labeling was seen ipsilaterally in the SC, and bilaterally in the thalamus with a clear ipsilateral predominance. The layers of the SC, where terminal labeling were observed, were considered to correspond to the intermediate and deep layers of the SC of common laboratory mammals, because the superficial layers of the SC are lacking in the mole. Terminal labeling was seen not only in the medial geniculate body (MG), a major auditory center in the thalamus, but also in the LG, a major visual center in the thalamus, where terminal labeling was observed throughout the entire LGd and LGv.

In nine animals a retrograde tracer, FR, was injected into the occipital, the parietal or the temporal lobe of the cerebral cortex. The LG neurons were labeled retrogradely with FR only in four animals receiving tracer injections into the occipital cortex. Even in the animals in which the largest number of FR-labeled cells was found in the LG, the FR labeled neurons in the LGd were small in number; instead, a large number of FR labeled cells were seen in the lateroposerior nucleus of the thalamus.

A characteristic transneuronal tracer, TTC, was injected into the eyeball in three animals. A considerable number of TTC labeled cells bodies were found contralaterally in the LGd and the pretectum of these animals. They were interpreted to have been labeled retrograde with the tracer taken up by axon terminals of the LG neurons projecting to the SCN, which was known to solely receive a substantial amount of the retinal projection in the mole. Because TTC does not undergo anterograde transneuronal transport, TTC-labeled cell bodies in the LG and the pretectum were not considered to have been labeled transneuronally through retinal fibers projecting to the LGd or the pretectum.

In these animals injected with WGA-HRP, a retrograde tracer, into the SC and the pretectum, many cells in the LGv, as well as in the thalamic reticular nucleus, were labeled retrogradely whereas no cells in the LGd were labeled. This indicates that neither the SC nor the pretectum is a subcortical target of the LGd. Thus, it was assumed that the SCN might be the main target of the subcortical projections from the LG in thew mole.

DISCUSSION

In *Spalax* , both 2-deoxyglucose studies and electrophysiologycal studies have shown that a fair-sized striate cortex and a distinguishable LGd are vigorously activated by auditory inputs (Bronchti *et al*., 1989; Heil *et al*., 1991), and that the primary source of the auditory inputs into the visual system is the IC (Doron and Wollberg, 1994). The present results also show that both the retinal and the IC projections terminate throughout the entire LG in the adult mole. According to Bronchti *et al*. (1991), retinal fibers projecting to the LG of *Spalax* retract progressively during the first week of postnatal development, and few, if any, retinal fibers are present in the LGd of adults. Thus, possible exuberant fibers from the IC have been considered to take over the retinal termination areas in the LG during development in *Spalax*. On the other hand, Cooper *et al*. (1993a,b) performed a rigorous study of the retinal projections in adult *Spalax* and reported that the LGd receives a strong retinal projection. In the mole, both the retinal and the IC fibers are now known to terminate in the LG, intermingled each other. The existence of direct projections from the IC to the LG may represent an ‘intermodal’

modification common to blind subterranean mammals.

In common laboratory mammals, the main subcortical afferent fibers to the primary visual and auditory areas in the cerebral cortex arise from the LG and the MG, respectively. The present results indicate that the LG and the MG of the mole also receive the retinal and the IC fibers, respectively, and transmit visual and auditory information from the peripheral sensory organs to the visual and auditory cortex, respectively. Thus, the functional significance of the direct projections from the IC to the LG in mole is still enigmatic. In mammals, the geniculo-hypothalamic projection from the intergeniculate leaflet to the SCN has been known to be subserve photoperiodic functions (Meijer and Reitveld, 1989; Card and Moore, 1989). The present study also revealed that the LG of the mole sends projections fibers to the SCN, as well as to the cerebral cortex. Although the intergeniculate leafleti is not discernible as an independent entity in the mole, neurons sending their axons to the SCN were identified within the LGd and the LGv without making a specific cell cluster. It is likely, therefore, that the auditory inputs from the IC conveyed through these LG neurons are targeted primarily to the SCN, which is selectively well developed among the visual centers in the mole.

In the frog, the hypothalamus receives bimodal projections from both auditory and visual sources, and the auditory information is important for reproductive behavior in parallel with the photoperiodic function which controls the circadian rhythms and sustains the seasonally reproductive response (Card and Moore, 1989; Allison and Wilczynski, 1994). In a similar strategy, the mole may use an auditory cue brought by the IC projections to the SCN for reproductive behavior such as ovulation in the female to be induced by seasonally mating calls of the male. This can be a simple and efficient strategy for the successful reproduction in the dark underground niche of the mole (Simpson, 1945).

BIBLIOGRAPHICAL REFERENCES

1. Allison JD, Wilczynski W (1994) Brain Beh. Evol.43:129-139.
2. Arboitiz F (1993) Med. Hypotheses 41:409-418.
3. Bronchti G, Heil P, Scheich H, Wollberg Z (1989) J. Comp. Neurol. 284:253-274.
4. Bronchti G, Rado R, Terkel J, Wollberg Z (1991) Dev. Brain Res. 58:159-170.
5. Card JP, Moore RY (1989) J. Comp. Neurol. 284:135-147.
6. Coles RB, Gower DM, Boyd PJ, Lewis DB (1982) J. Exp. Biol. 101:337-341.
7. Cooper HM, Herbin M, Nevo E (1993a) J. Comp. Neurol. 328:313-350.
8. Cooper HM, Herbin M, Nevo E (1993b) Nature 361:156-159.
9. Doron N, Wollberg Z (1994) NeuroReport 5:2697-2701.
10. Hardy H, Heimer L, Switzer R (1976) Neurosci. Lett. 3:1-5.
11. Heil P, Bronchti G, Wollberg Z, Scheich H (1991) NeuroReport 2:735-738.
12. Henson OW (1988) Handbook of Sensory Physiology. Vol. V-1 Auditory System, New York: Willey, p96.

13. Kudo M, Nakamura Y. Moriizumi T (1988) Neurosci. Lett. 93:176-180.
14. Kudo M, Yamamoto M, Nakamura Y (1991) Brain Beh. Evol. 38: 332-338.
15. Kuypers HGJM, Huisman AM (1984) Adv.Cell Neurobiol. 5:307-327.
16. Lund RD, Lund JS (1965) Exp. Neurol. 13:302-316.
17. Manning YA, Erichisen JT, Evinger C (1990) Neuroscience 34:251-263.
18. Meijer JH, Reitveld WJ (1989) Physiol. Rev. 69:671-707.
19. Mesulam MM (1978) J. Histochem. Cytochem. 26:106-117.
20. Necker R, Rehkamper G, Nevo E (1992) NeuroReport. 3:505-508.
21. Nowak RM, Paradiso JL (1983) Walker's Mammals of the World. Baltimore: Johns Hopkins University Press, pp. 156-157.
22. Quilliam TA (1966) J. Zool. 146:76-88.
23. Simpson GG (1945) Bull. Am. Museum Natural History, 85:1-350.
24. Voneida TJ, Fish SE (1984) Am. Zool. 24:775-782.