Afferent Projections to the Cingulate Cortex in Albino Rats: a Study with a Retrograde Labeling Method Using Fluoro-Gold

Ahmed, A.K.M. Farid
Dong, Kai
Sugioka, Kozo [et. al.]

(Citation)
The Kobe journal of the medical sciences,41(6):247-255

(Issue Date)
1995-12

(Resource Type)
departmental bulletin paper

(Version)
Version of Record

(URL)
https://hdl.handle.net/20.500.14094/E0000944

Kobe J. Med. Sci. 41, 247/255, December 1995

AFFERENT PROJECTIONS TO THE CINGULATE CORTEX IN ALBINO RATS: A STUDY WITH A RETROGRADE LABELING METHOD USING FLUORO-GOLD

A. K. M. Farid AHMED, Kai DONG, Kozo SUGIOKA, and Takashi YAMADORI

First Division, Department of Anatomy, Kobe University School of Medicine

INDEXING WORDS

afferent; cingulate; retrograde fluorescent tracer; rat

SYNOPSIS

We studied the neuronal populations that project their axons to the cingulate cortex in albino rats using the retrograde fluorescent dye of 4% Fluoro-Gold injected into the anterior, middle and posterior portions of the cingulate cortex. The result showed that the following ipsilateral structures are sending fibers to these three portions: the prefrontal cortex, frontoparietal motor cortex, indusium griseum, dorsal endopiriform nucleus, lateral part of medial mammillary nucleus, nuclei of diagonal band of Broca, anterior pretectum, anterior part of caudate-putamen, hippocampal formation, anteroventral, anteromedial, lateroposterior, ventroposterior and dorsomedial thalamic nuclei. The anterior portion of the cingulate cortex receives inputs from the following ipsi- and contralateral structures: the accessory olfactory bulbs, anterior olfactory nuclei, middle and posterior portions of the cingulate cortex. The middle cingulate cortex receives fibers only from the ipsilateral dorsal part of the lateral septal nucleus in addition from the ipsi- and contralateral anterior and posterior portions of the cingulate cortex. While the posterior portion of the cingulate cortex receives separate inputs from the following ipsilateral structures: anterodorsal thalamic nucleus, temporal cortex, entorhinal cortex, areas 17 and 18, as well as from the ipsi- and contralateral anterior and middle portions of the cingulate cortex. The present study shows that the cingulate cortex receives various kinds of inputs from the other parts of the brain which are involved in emotion, memory, vision and motion, and also suggests that there are differences in afferent projections among the anterior, middle and posterior portions of the cingulate cortex.

Received for publication: December 18, 1995

Authors' names in Japanese: A.K.M.ファリド アハメド，董　凱，杉岡幸三
山鳥　崇

INTRODUCTION

The Papez's report (24) first linked the cingulate cortex with the theory of emotionality. A series of studies in a variety of species documented the connections of the cingulate cortex with the neocortex (1, 23, 32), nucleus of the diagonal band (6, 19, 31), thalamus (1, 3, 5, 9, 10, 13, 18, 21, 28, 29), basolateral nucleus of the amygdala (1, 23, 27, 35), claustrum (4), lateral septum (16), entorhinal cortex (1), subicular complex of the hippocampal formation (1, 20, 30, 32, 33), brain stem including the medial raphe, dorsal raphe, locus cerulus, substantia nigra, and anterior ventral tegmentum (3, 6, 8, 15, 26), caudate-putamen (1) and ipsi- and contralateral cingulate cortices (1, 2, 33, 35, 36).

Despite this wealth of information, there are still significant gaps in our knowledge of input to the cingulate cortex particularly in the topographical input to the different parts of the cingulate cortex. This study addresses this particular question utilizing a well-known retrograde fluorescent tarcer, Fluoro-Gold (FG), injected into the anterior/middle/ or posterior part of the cingulate cortex in the albino rat.

MATERIALS AND METHODS

Eighteen adult albino rats (Wistar, Japan Clea) of either sex that weighed 300-400 g were used. While the animal was under deep anesthesia (10% chloral hydrate, 0.3 mg/kg; i.p.), the head was stereotaxically fixed (Narishige, SR-6). An incision was made in the midline of the scalp to expose the area of the bregma and the sagittal sutures. Small holes were then drilled through the frontal or parietal bone with a dental hand drill at sites corresponding to the anterior, middle or posterior part of the right cingulate cortex. The site of the hole was plotted according to coordinates from Paxinos and Watson's rat Brain Atlas (25) in order to approach the anterior, middle and posterior parts of the cingulate cortex.

The rats were divided into three groups. The first group received the dye, Fluoro-Gold (FG) in the anterior part, second group in the middle part and the third group in the posterior part of the right cingulate cortex iontophoretically via a fine glass capillary (tip diameter about 50μm). The iontophoretic parameters used were 4-5 min of 4-5μA of pulse positive current delivered via a handmade transkinetics high-voltage precision current source. Postoperatively all of the animals were left in a dark room. After survival periods ranging from 48 to 72 h, the rats were put under deep anesthesia (10% chloral hydrate, 0.3 mg/ kg; i.p.), then perfused with 10% formol-saline after a vascular rinse with normal saline. The brain was then isolated and excised which was finally immersed in 10% formol-saline for several hours and sectioned serially on a cryostat in the coronal plane at a thickness of 40 μm. Every third section was mounted on a glass slide. The exact site of injection, as well as the afferent projection to the different parts of the cingulate cortex from the other cortical region within each structure, was confirmed by fluorescence microscopy (Olympus AX 80) using filter system UV.

RESULTS

1) *Localization of injected tracer*

Experiments were considered successful only if the injection adequately labeled the target area in the cingulate cortex without any obvious involvement of the other adjoining areas or the contralateral cortex. Figure 1A shows the injection site and the extent of labeling; where the tracers labeled the intended targets fully and exclusively.

2) *Injection to the anterior portion of the cingulate cortex*

Ipsi- and contralateral accessory olfactory bulbs, anterior olfactory nuclei, middle and posterior portions of the cingulate cortex had been labeled after injection into the anterior portion of the cingulate cortex. The ipsilateral prefrontal cortex, frontoparietal motor cortex, indusium griseum (Fig. 1B), dorsal endopiriform nucleus, lateral part of medial mammillary nucleus, anteroventral, anteromedial and lateroposterior thalamic nuclei (Fig. 1F), vertical and horizontal limbs of diagonal band nucleus (Fig. 1G), anterior pretectal area, anterior portion of caudate-putamen, hippocampal formation, ventroposterior and dorsomedial thalamic nuclei were also labeled after the injection into the anterior portion.

3) *Injection to the middle portion of the cingulate cortex*

The ipsilateral prefrontal cortex, frontoparietal motor cortex, indusium griseum (Fig. 1B), dorsal endopiriform nucleus, lateral part of medial mammillary nucleus, anterodorsal (Fig. 1D), anteroventral, anteromedial, dorsomedial (Fig. 1 E) and lateroposterior thalamic nuclei (Fig. 1F), dorsal lateral septal nucleus (Fig. 1 C), vertical and horizontal limbs of diagonal band nucleus (Fig. 1G), anterior pretectal area, anterior portion of caudate-putamen, hippocampal formation, ventroposterior and dorsomedial thalamic nuclei as well as from the ipsi- and contralateral anterior and posterior portions of the cingulate cortex had been labeled after the injection into the middle portion of the cingulate cortex.

4) *Injection to the posterior portion of the cingulate cortex*

The ipsilateral prefrontal cortex, frontoparietal motor cortex, indusium griseum (Fig. 1B), dorsal endopiriform nucleus, lateral part of medial mammillary nucleus, anterodorsal (Fig. 1D), anteroventral, anteromedial and lateroposterior thalamic nuclei (Fig. 1F), ventral and horizontal limbs of diagonal band nucleus (Fig. 1G), anterior pretectal area, anterior portion of caudate-putamen, hippocampal formation, ventroposterior and dorsomedial thalamic nuclei, temporal cortex, entorhinal cortex, areas 17 and 18, as well as from the ipsi- and contralateral anterior and middle portions of the cingulate cortex had been labeled after the injection into the posterior portion of the cingulate cortex.

Representative example in Fig. 2 diagrammatically summarizes the different areas of the brain which projects to the cingulate cortex.

DISCUSSION

We studied the afferent projections to the cingulate cortex in the albino rat with a retrograde tracing method. We found that several ipsilateral subcortical structures send their axons almost

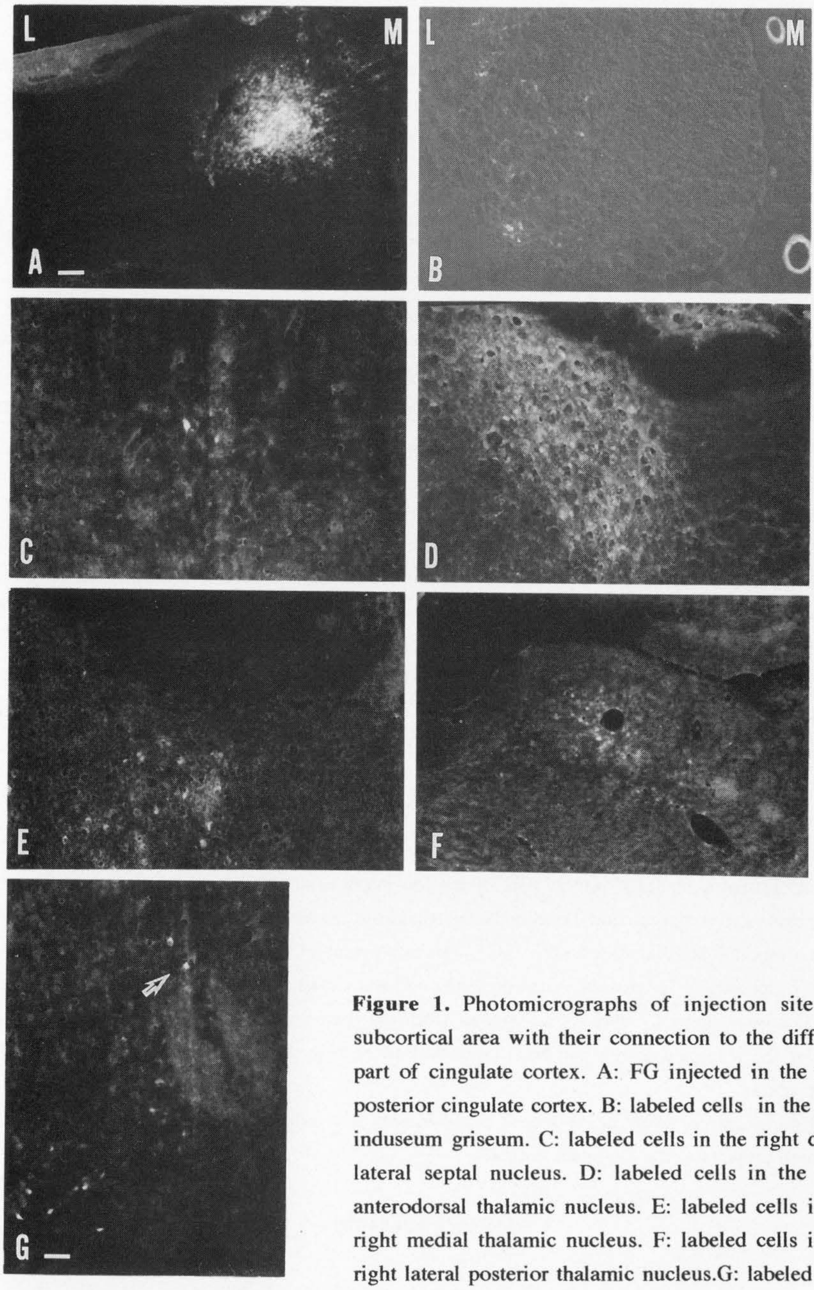

Figure 1. Photomicrographs of injection site and subcortical area with their connection to the different part of cingulate cortex. A: FG injected in the right posterior cingulate cortex. B: labeled cells in the right induseum griseum. C: labeled cells in the right dorsal lateral septal nucleus. D: labeled cells in the right anterodorsal thalamic nucleus. E: labeled cells in the right medial thalamic nucleus. F: labeled cells in the right lateral posterior thalamic nucleus.G: labeled cells in the right vertical (arrow) and horizontal limbs of diagonal band nucleus. M: medial, L: lateral, Bar scale A = 0.1 mm, B - G Bar scale = 0.25 mm.

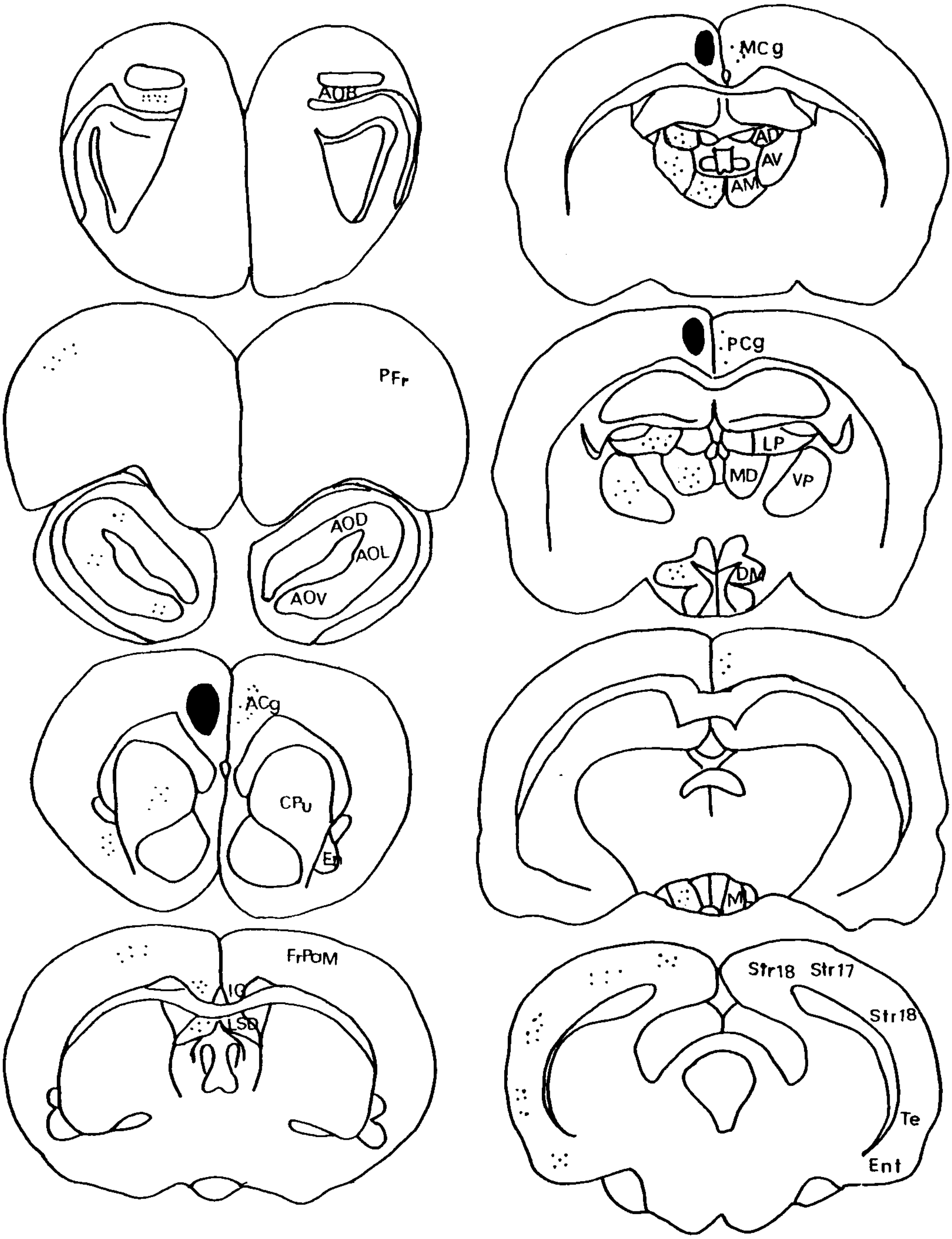
AOB
MCg
AD
AV
AM
PFr
PCg
LP
MD
VP
AOD
AOL
AOV
DM
ACg
CPu
En
FrPaM
IG
LSD
Str18
Str17
Str18
Te
Ent

Figure 2. Diagrammatical summarization of different areas of the brain which project to the cingulate cortex. ACg: anterior cingulate cortex, AD: anterodorsal thalamic nucleus, AM: anteromedial thalamic nucleus, AOB: accessory olfactory bulb, AOD: Anterior olfactory nucleus, dorsal part, AOL: Anterior olfactory nucleus, lateral part, AOV: Anterior olfactory nucleus, ventral part, AV: anteroventral thalamic nucleus, Cpu: caudate-putamen, DM: dorsomedial hypothalamic nucleus, En: endopiriform nucleus, Ent: entorhinal cortex, FrPaM: frontoparietal motor cortex, IG: indoseum griseum, LP: lateral posterior thalamic nucleus, LSD: lateral septal nucleus, dorsal part, MCg: medial cingulate cortex, MD: mediodorsal thalamic nucleus, ML: lateral part of medial mammillary nucleus, PCg: posterior cingulate cortex, PFr: prefrontal cortex, Str: striate cortex, Te: temporal cortex, VP: ventroposterior thalamic nucleus.

equally to the cingulate cortex, while there are also distinct differences in the afferent projections among the anterior, middle and posterior portions of the cingulate cortex. It has been accepted that the main functions of the cingulate cortex are involved in emotion (24) and memory based behavior (12, 17). The findings of the present study suggests that these functions of the cingulate cortex are widely affected by various kinds of inputs from the several parts of the brain, and also that each portion of the cingulate cortex have different functions through the different neuronal connections with the specific structures in the brain.

The present study showed that several brain structures such as prefrontal cortex, frontoparietal motor cortex, indusium griseum, dorsal endopiriform nucleus, lateral part of the medial mammillary nucleus, vertical and horizontal limbs of diagonal band nucleus, anterior pretectal area, anterior caudate-putamen, hippocampal formation as well as the anteroventral, anteromedial, lateroposterior, ventroposterior and dorsomedial thalamic nuclei ipsilaterally connect almost equally with the anterior, middle and posterior portions of the cingulate cortex. Close relationships between the cingulate cortex and these structures were also reported by Finch et al. (11) and Domesick (9). Of these structures, it is said that prefrontal cortex, anterior and dorsomedial thalamic nuclei, hippocampal formation and lateral part of the medial mammillary nucleus are involved in memory and emotion, and lateroposterior thalamic nucleus in vision. These findings supplement the ideas that the function of the cingulate cortex relates with emotion, memory and vision through these brain structures.

In the present study, we found also that there were differences in the afferent projections among the anterior and posterior portions of the cingulate cortex. The anterior portion of the cingulate cortex receive axons from the ipsi- and contralateral accessory olfactory bulbs, anterior olfactory nuclei, and midlle and posterior portions of the cingulate cortex in addition to the structures projecting to the other portions of the cingulate cortex. Physiological studies suggest that the accessory olfactory bulbs, anterior olfactory nuclei, hippocampal formation and dorsomedial thalamic nucleus are involved in memory-based behavior, control of spatial orientations (12, 17) and the caudate-putamen is involved in the control of skeletal muscle activity (7). These findings of the present study suggest that the anterior portion of cingulate cortex might be closely related with those structures in yielding its function. In contrast, the afferent projection to the posterior portion of the cingulate cortex was quite different from that to the anterior portion. This differences was also pointed out by several authors in the rat, cat, and monkey (1, 10, 11, 18, 21, 28, 30, 35). In our materials, the anterodorsal thalamic nucleus, temporal cortex, entorhinal cortex, areas 17 and 18 exhibited distinct connections mostly with the posterior cingulate cortex. Interactions between the temporal cortex and posterior portion of the cingulate cortex were also reported by Finch et al. (11) and Vogt and Pandya (34). The relationship between the visual system and limbic system was also suggested by Itaya et al. (14). Although the anterodorsal thalamic nucleus projects from the middle to the posterior cingulate cortex, the projection was stronger to the posterior portion. This projection has been reported by many authors (3, 11, 22). These findings taken together suggest that the posterior portion of the cingulate cortex has relationship with memory and vision. The middle portion of the cingulate cortex receives the afferent from the dorsal part of the lateral septal nucleus as well as from the anterodorsal thalamic nucleus. This portion is thought to be related with the emotion and memory.

The projections from the ipsi- and contralateral anterior and middle portions to the posterior portion of the cingulate cortex were also noted in the present study. These findings suggest that though each portion of the cingulate cortex functions differently, the function of each portion of the cortex might be regulated by each other by the neuronal connections within the cingulate cortex.

As discussed in the present study, the cingulate cortex receives inputs from a number of structures of the brain, and there are regional differences in functions within the cingulate cortex. There may be much more afferent projections to this region, in addition to those described in the present study. To elucidate the functions of the cingulate cortex completely, further experiments should be performed on the other projections to the cingulate cortex, the cell type which projects into the cortex, physiological properties of inputs and so on.

REFERENCES

1. Baleydier, C. and Mauguire, F. : Brain 1980. 103. 525/554. The duality of the cingulate gyrus in monkey.
2. Bassett, J. L. and Berger, T. W. :Brain Res. 1982. 248. 371/376. Associational connections between the anterior and posterior cingulate gyrus in rabbit.
3. Beckstead, R. M. : J. Comp. Neurol. 1976. 166. 403/416. Convergent thalamic and mesencephalic projections to the anterior medial cortex in the rat.
4. Berger, T. W., Bassett, J. L., and Semple-Rowland, S.L. : Soc. Neurosci. Abstr. 1981. 7. 886. Neocortical input to entorhinal and cingulate cortices is relayed by the claustrum.
5. Berger, T. W., Milner, T. A, Swanson, G. W., Lynch, G. S., and Thompson, R. F. : Brain Res. 1980. 201. 411/417. Reciprocal anatomic connections between anterior thalamus and cingulate-retrosplenial cortex in the rabbit.
6. Bobillier, P., Seguin, F. P., Salvert, D., Touret, M., and Jouvet, M. : Brain Res. 1976. 113. 449/486. The raphe nuclei of the cat brain stem: a topographical atlas of their efferent projections as revealed by autoradiography.
7. Campbell, B. A. and Lynch, G. S. : J. Comp. Physiol. 1969. 67 15/22. Cortical modulation of

spontaneous activity during hunger and thirst.

8. Conrad, L. C., Leonard, C. M., and Pfaff, D. W. : J. Comp. Neurol. 1974. 156. 179/206. Connections of the median and dorsal raphe nuclei in the rat: an autoradiographic and degeneration study.
9. Domesick, V. B. : Brain Res. 1969. 12. 296/320. Projections from the cingulate cortex in the rat.
10. Domesick, V. B. : Brain Behav. Evol. 1972. 6. 457/483. Thalamic relationships of the medial cortex in the rat.
11. Finch, D. M., Derian, E. L., and Babb, T. L. : Exp. Neurol. 1984. 83. 468/485. Afferent fibers to rat cingulate cortex.
12. Goldman-Rakic, P. S., Isseroff, A., Schwartz, M. L., and Bugbee, N. M. : In: Mussen, P., ed., Hand book of child psychology: Biology and Infancy Development (Wiley, New York). 1983. 281/344. The neurobiology of cognitive development.
13. Herkenham, M. : J. Comp. Neurol. 1978. 177. 589/610. The connections of the nucleus reunions thalami: evidence for a direct thalamo-hippocampal pathway in the rat.
14. Itaya, S.K., Van Hoesen, G. W., and Jenq, C. -B. : Brain Res. 1981. 226. 33/42. Direct retinal input to the limbic system of the rat.
15. Jones, B. E. and Moore, R. Y. : Brain Res. 1977. 127. 25/53. Ascending projections of the locus coeruleus in the rat. II. Autoradiographic study.
16. Kemper, T. L., Wright, S.J., Jr., and Locke, S. : J. Comp. Neurol. 1972. 146. 465/478. Relationship between the septum and the cingulate gyrus in macaca mulatta.
17. Kolb, B., Sutherland, R. J.,and Whishaw, I. Q. : Behav. Neurosci. 1983. 97. 13/27. A Comparison of the contributions of the frontal and parietal association cortex to spatial localization in rats.
18. Krettek, J. E. and Price, J. L. : J. Comp. Neurol. 1977. 171. 157/192. The cortical projections of the mediodorsal nucleus and adjacent thalamic nuclei in the rat.
19. Lewis, P. R. and Shute, C. C. D. : Brain 1967. 90. 521/540. The cholinergic limbic system: projections to hippocampal formation, medial cortex, nuclei of ascending cholinergic reticular system and the subfornical organ and supra-optic crest.
20. Meibach, R. C. and Siegel, A. : Exp. Neurol. 1977. 57. 264/274. Subicular projections to the posterior cingulate cortex in rats.
21. Niimi, K., Niimi, M., and Okada, Y. : Brain Res. 1978. 145. 225/238. Thalamic afferents to the limbic cortex in the cat studied with the method of retrograde axonal transport of horseradish peroxidase.
22. Olson, C.R. and Musil, S.R. : J. Comp. Neurol. 1992. 324. 237/260. Topographic organization of cortical and subcortical projections to posterior cingulate cortex in the cat: Evidence for somatic, ocular, and complex subregions.
23. Pandya, D. N., Van Hoesen, G. W., and Mesulam, M. -M. : Exp. Brain Res. 1981. 42. 319/330. Efferent connections of the cingulate gyrus in the rhesus monkey.
24. Papez, J. W. : Arch. Neurol. Psychiatry 1937. 38. 725/743. A proposed mechanism of emotion.

25. Paxinos, G. and Watson, C. : The Rat Brain in Stereotaxic Coordinates. 2nd ed. (Academic Press, Sydney). 1986. 25/45.
26. Pickel, V. M., Segal, M., and Bloom, F. E. : J. Comp. Neurol. 1974. 155. 15/42. A radioautographic study of the efferent pathways of the nucleus locus coeruleus.
27. Powell, E. W. : Anat. Rec. 1978. 190. 783/794. The cingulate bridge between allocortex, isocortex and thalamus.
28. Robertson, R. T. and Kaitz, S. S. : J. Comp. Neurol. 1981. 195. 501/525. Thalamic connections with limbic cortex. I. Thalamocortical projections.
29. Rose, J. E. and Woolsey, C. N. : J. Comp. Neurol. 1948. 89. 279/347. Structure and relations of limbic cortex and anterior thalamic nuclei in rabbit and cat.
30. Rosene, D. L. and Van Hoesen, G. W. : Science 1977. 198. 315/317. Hippocampal efferents reach widespread areas of cerebral cortex in rhesus monkey.
31. Sripanidkulchai, K. and Wyss J. M. : Soc. Neurosci. Abstr. 1982. 8. 217. Efferent projections of the nucleus of the diagonal band of brocha to the olfactory bulb and midline cortex.
32. Swanson, L. W. and Cowan, W. M. : J. Comp. Neurol. 1977. 172. 49/84. An autoradiographic study of the efferent connections of the hippocampal formation in the rat.
33. Vogt. B. A. and Miller, M. W. : J. Comp. Neurol. 1983. 216. 192/210. Cortical connections between rat cingulate cortex and visual, motor, and postsubicular cortices.
34. Vogt, B. A. and Pandya, D. N. : J. Comp. Neurol. 1987. 262. 271/289. Cingulate cortex of the rhesus monkey: II. Cortical afferents.
35. Vogt, B. A., Rosene, D. L., and Pandya, D. N. : Science 1979. 204. 205/207. Thalamic and cortical afferents differentiate anterior from posterior cingulate cortex in the monkey.
36. Vogt, B. A., Rosene, D. L., and Peters. A. : J. Comp. Neurol. 1981. 201. 265/283. Synaptic termination of thalamic and callosal afferents in cingulate cortex of the rat.