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Neurons in the ventral tegmentum have separate populations projecting to telencephalon and inferior olive, are histochemically different, and may receive direct visual input

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The connections of the ventral tegmental area (VTA) and medial terminal nucleus (MTN) of the accessory optic system were studied in the albino rat. Using injection of two fluorescent retrograde tracers it was found that individual neurons of the VTA project to frontal cortices or the inferior olive but not both structures. Using combined retrograde fluorescent tracers and glyoxylic acid histochemistry, it was found that although a third of the cells projecting to frontal cortex contained catecholamine, none of the cells projecting to the inferior olive contained catecholamine. Thus, these portions of the ascending and descending VTA systems are independent. In addition, using injections of the anterograde transneuronal tracer [^{3}H]adenosine into one eye, it was found that cells in the VTA, as well as the MTN, contained the tracer. Therefore, there is a basis for direct retino-mesentelencephalic pathways through the VTA.

The ventral tegmental area (VTA) is a functionally and anatomically diverse region of the midbrain. The VTA contains both dopaminergic (A10 cell group) and non-dopaminergic neurons that project to telencephalic and brainstem areas[6,22,23,28]. It is not known whether ascending and descending projections arise from the same cells, although it is known that the ascending projections of the VTA are from poorly collateralized neurons[4,5,26], whereas axons of the adjacent medial substantia nigra are highly collateralized to the same structures[4,5]. The VTA has also been the focus of a second major research interest involving the transfer of visual information to the flocculus via the accessory optic system[2,16,25]. As diagrammed schematically in Fig. 1, retinal ganglion cells project to the contralateral medial terminal nucleus (MTN), located at the ventrolateral border of the VTA[11,13,14]. The MTN, in turn, projects to the ipsilateral anterior dorsal cap of the inferior olive and contralateral VTA adjacent to the MTN, an area that has been called the VTRZ (visual tegmental relay zone)[7]. The VTRZ also projects to the ipsilateral dorsal cap of the inferior olive[17]. The dorsal cap region conveys visual information related to vertical eye movements to the ipsilateral flocculus[3], which then projects to the ipsilateral vestibular nuclei[15,25,27]. The vestibular nuclei affect eye movements via projections to cranial nuclei III, IV and VI. With respect to the role of the descending VTA projections in eye movements, it is interesting to note that some ascending VTA projections are to the frontal eye fields of the cat[19].

The presence of both mesotelencephalic and mesobulbar VTA systems, the former involved in the limbic-striatal functions and the latter involved in visuovestibular functions, has prompted us to investigate the possible overlap or interrelationships between these VTA projection systems. In the present study we were interested in addressing the following questions: (1) Do individual neurons of the VTA innervate both telencephalon (striatum, cortex, limbic system) and brainstem (inferior olive)? (2) Are the VTA neurons involved dopaminergic? (3) Could the VTA neurons projecting to the telencephalon and/or brainstem receive direct retinal input?

Twenty-eight female albino rats of the Sprague–Dawley strain were used in the experiments. Axon collateralization of VTA neurons (Group A), was determined in 6 animals by the use of the double-label-

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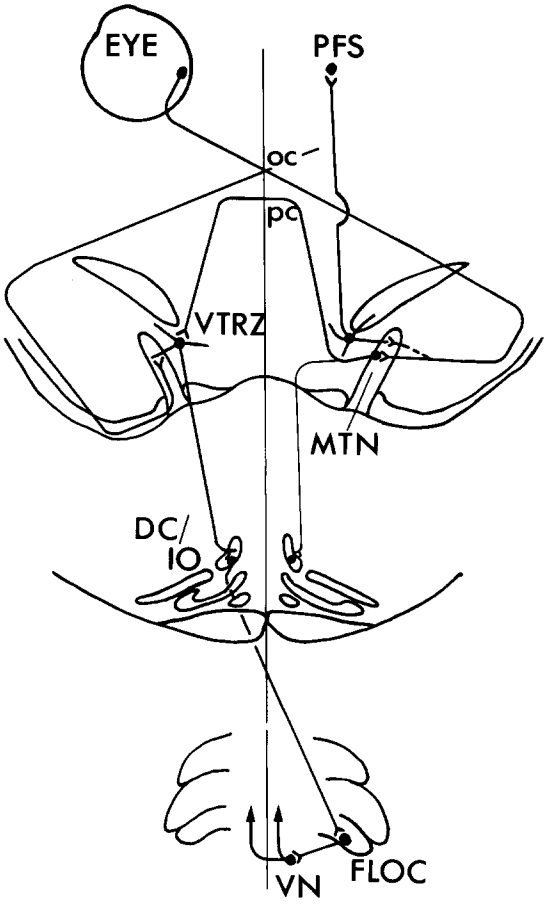

Fig. 1. Diagram of the visual and mesotelencephalic pathways involved in this study (for review see ref. 24). Retinal ganglion cells of the left eye project to the contralateral (approx. 98%) medial terminal nucleus (MTN), crossing in the optic chiasm (OC). The MTN has a minor direct ipsilateral projection to the dorsal cap of the anterior half of the inferior olive (DC/IO) and a strong projection through the posterior commissure to the contralateral visual tegmental relay zone (VTRZ) located primarily in, and dorsal to, the subnucleus parabrachialis pigmentosus of the VTA. The VTRZ cells on the left side may also receive a direct retinal input to their dendrites from the right eye by virtue of their dendrites invading the neuropil of the MTN. In addition, other cells of the VTA which project to cortical and subcortical regions of the 'prefrontal system' (PFS) may also receive a similar retinal input on their dendrites located in the MTN (right side of figure). The connections of the DC/IO are further illustrated at the bottom of the figure. The DC/IO projects to the contralateral flocculus (FLOC) which, in turn, projects to sectors of the vestibular nuclei (VN). The VN project to cranial nerve nuclei III, IV and VI (probably on both sides of the brain) which then innervate extraocular muscles (not shown).

ing technique with the fluorescent dyes True Blue, Nuclear Yellow and Propidium Iodide[1,4]. In order to study the possible dopaminergic content of VTA neurons (Group B), the fluorescent dye technique was used in 8 animals in conjunction with the glyoxylic acid-cryostat technique[12]. In order to study the possibility that VTA neurons which project to the inferior olive or telencephalon also receive retinal input (Group C)[9], the [^{3}H]adenosine anterograde transneuronal transport technique[21] was used in 4 animals.

In Group A injections of either 0.05 μl (small injection) or 0.15 μl (large injection) of one of the fluorescent dyes into the inferior olive resulted in retrograde

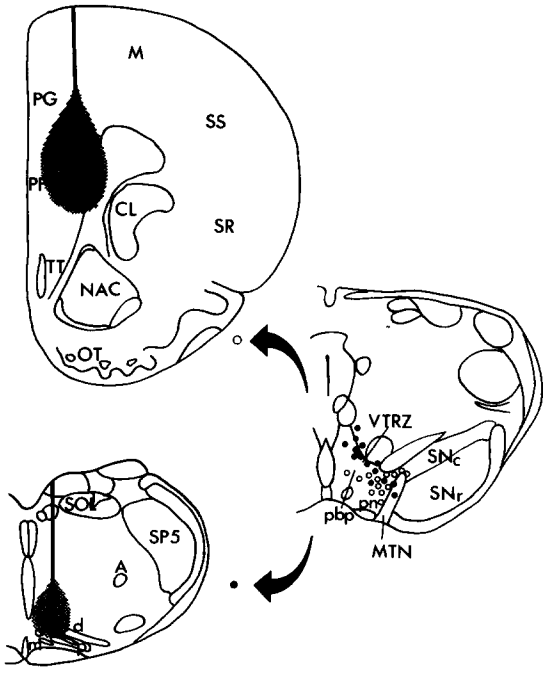

Fig. 2. Drawings of coronal sections of rat brain illustrating location of fluorescent tracer injections into the prefrontal cortex (PF) and inferior olive and subsequent retrograde labeling in the ventral tegmentum. Open circles represent cells projecting to cortex, filled circles represent cells projecting to inferior olive. Each dot represents 3 labeled neurons in a 20 μm section. The injection sites are an average size of a fluorescent tracer injections for these experiments. In retrograde tracer double-labeling (fluorescent dyes) experiments, no cells were found to project to both cortex and inferior olive. In combined retrograde tracer/glyoxylic acid fluorescence studies, no cells projecting to inferior olive were found to contain catecholamine, whereas over one-third of the cells projecting to prefrontal cortex contained catecholamine. Abbreviations: PF, PG, M, SS, SR = prefrontal, pregenual, motor, somatosensory, suprarhinal cortices; OT = olfactory tubercle; CL = claustrum; TT = tenia tecta; NAC = nucleus accumbens; SOL = solitary nucleus; A = nucleus ambiguus; SP5 = spinal nucleus V; d, p, m = dorsal, principal and medial nuclei of the inferior olive; MTN = medial terminal nucleus; SN_c, SN_r = pars compacta and pars reticulata of the substantia nigra; pbp, pn = nucleus parabrachialis and paranigralis of the ventral tegmental area; VTRZ = visual tegmental relay zone of the ventral tegmental area.

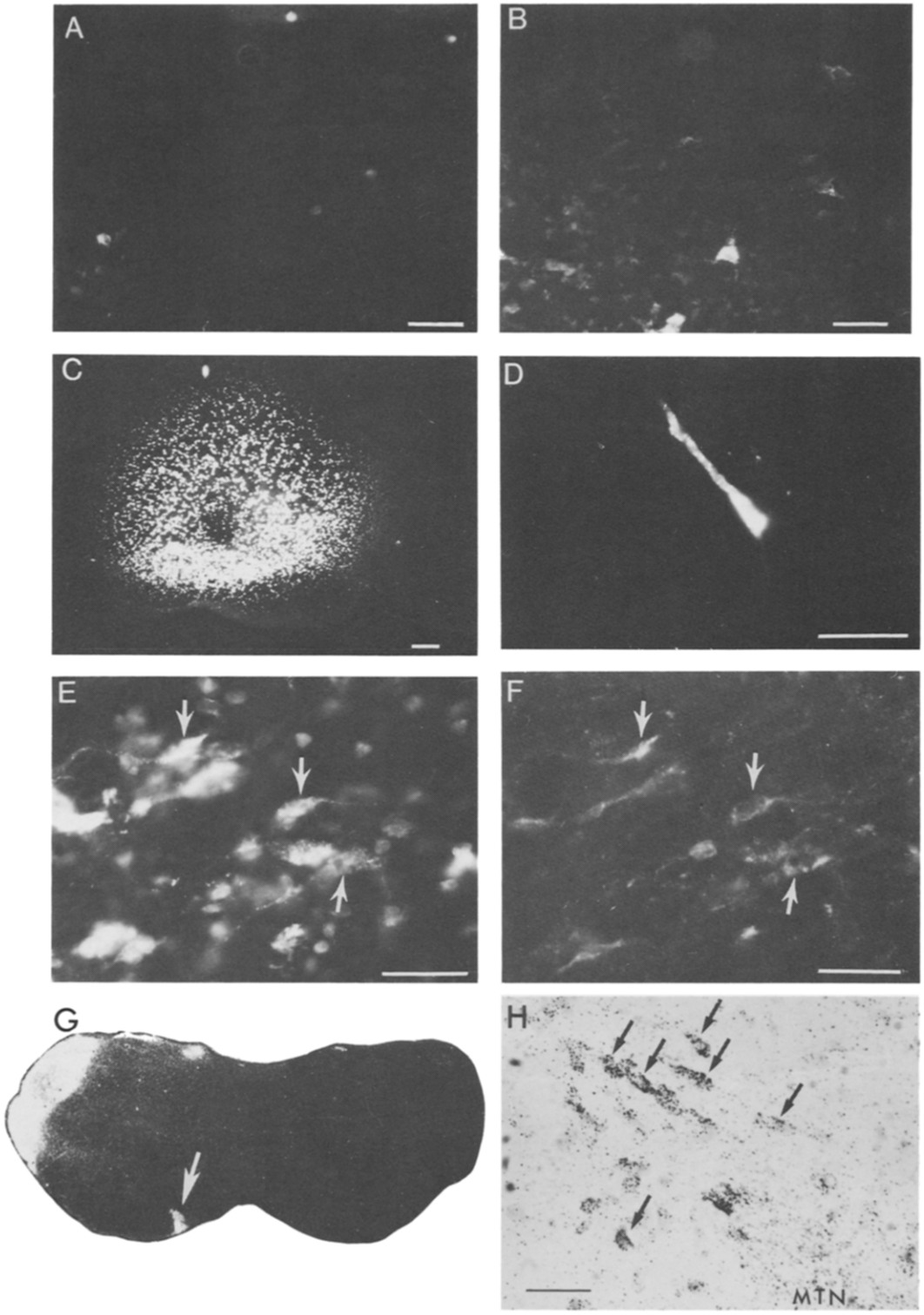

Fig. 3. Photomicrographs of fluorescence and autoradiographic data. A and B: single-labeled cells in the VTRZ region of the dorsal VTA following a 0.15 μl injection of Nuclear Yellow (NY) into the prefrontal cortex and a 0.05 μl injection of Propidium Iodide (PI) into the dorsal cap region of the anterior inferior olive. The UV filter allows visualization of NY-labeled cells (A) while the rhodamine filter allows visualization of PI-labeled cells (B) in the same field. C and D: injection of 0.15 μl of TB into the dorsal cap region (dc) of

labeling of neurons in the ventromedial tegmentum (Fig. 2). In cases where injections were restricted mainly around the dorsal cap region of the inferior olive (Fig. 2), neurons were retrogradely labeled in an arc of the ipsilateral nucleus parabrachialis pigmentosus of the dorsal VTA (Fig. 3C and D) extending from the region medial to the red nucleus and ventrolateral to the interstitial nucleus of Cajal, to the medial third of the medial lemniscus. Several neurons were also labeled in the medial terminal nucleus. With larger injections into the inferior olive and adjacent tegmentum, cells were also retrogradely labeled in the periaqueductal gray, interstitial nucleus of Cajal, and region ventral and adjacent to the fasciculus retroflexus. In the same animals, another fluorescent dye was injected into the medial caudate-putamen, nucleus accumbens, septum, olfactory tubercle, or areas 24b (PG = pregenual) and 32 (PF = prefrontal) of frontal-cingulate cortex (Fig. 2). Cells were retrogradely labeled in a topographical manner (c.f. ref. 6) in the VTA and substantia nigra. Although these single labeled cells were intermixed with single labeled cells projecting to the inferior olive, in no case were cells double-labeled with both dyes (Fig. 3A and B). This further substantiates the general finding that VTA neurons do not have highly branched axons[4,5,26] and also argues for an anatomical separation of the mesotelencephalic and mesobulbar projection systems.

The Group B experiments were done to determine the possible dopaminergic nature of the VTA projections, and to substantiate the results of the Group A experiments by repeating similar combinations of injections. As in Group A, there were no cells in the VTA that were double-labeled with the fluorescent dyes injected into the forebrain or inferior olive. Furthermore, although approximately 40% of the VTA neurons innervating the forebrain also demonstrated bluish-green catecholamine fluorescence (Fig. 3E and F), none of the VTA neurons innervating the inferior olive were catecholaminergic. This finding further argues for separate ascending and descending projecting populations of VTA neurons.

In the Group C animals, 1 μl of [^{3}H]adenosine (100 Ci/μl) was injected into the vitreous chamber of the left eye. Following a 7–14-day survival, the animals were processed for anterograde transneuronal transport of [^{3}H]adenosine metabolites to the ventromedial tegmentum (Fig. 3G and H). Silver grains were located over cells and neuropil in the medial terminal nucleus, as well as numerous cells in the adjacent VTA. These results suggest that VTA neurons may receive direct retinal input. The retinal input may reach VTA neurons via dendrites located within the MTN[8].

The results of the first experiments provide evidence, albeit negative, that the ascending mesotelencephalic neurons of the VTA are anatomically distinct from, but intermixed with, VTA neurons projecting to the inferior olive. While the ascending projections are predominantly catecholaminergic, the descending VTA projection to the inferior olive are not catecholaminergic. While these latter findings are not surprising, they do demonstrate that a 'reticular-like' area such as the VTA may contain intermixed subpopulations of neurons with quite distinct and separate afferents, efferents and functions. Thus, the ascending VTA systems related to motor, limbic and cortical function[18] may be synaptically independent from the descending system to the inferior olive, which has been proposed to be involved in the visual-vestibular coordinate system of signaling self-motion of the animal[24,25]. It is interesting that the visual projections from another mesencephalic region, the pretectal complex to inferior olive also arise from populations of pretectal cells separate from those ascending to the limbic thalamus[20].

The [^{3}H]adenosine experiments provide some evidence that the VTA may receive direct visual input from retinal ganglion cells. The retinorecipient VTA cells could be relay cells of the VTRZ projecting to the inferior olive, or may relay visual activity directly

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the inferior olive (C) and retrogradely labeled neuron in the VTRZ (D) following the injection. E and F: double-labeled neurons in the lateral VTA-medial substantia nigra junction just dorsal to the MTN following a 0.15 μl injection of PI into the prefrontal cortex. This region contains the largest population of cells double-labeled with both PI (E) and catecholamine histofluorescence (F). Some double-labeled cells are indicated by arrows. G and H: autoradiograms of midbrain region following injection of [^{3}H]adenosine into the right eye. Fourteen days survival. A low-power dark-field photomicrograph (G) shows silver grains over the MTN (arrow) as well as other retinal targets of the pretectum and lateral geniculate nuclei. At higher power (H), silver grains can be seen concentrated over VTA cells (arrows) dorsal to the MTN. Marker bars = 50 μm.

to the prefrontal system including, for example, the caudate-putamen, amygdala or frontal-cingulate cortices[6]. A retino-mesotelencephalic projection to cortical areas 24b and 32 would be interesting because these cortical regions could be the equivalent of the primate frontal eye fields in the rat[10]. The existence of this retinal pathway is presently being investigated with double-labeling techniques.

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1 Bentivoglio, M., Kuypers, H. G. J. M., Catsman-Berrevoets, C. E. and Dann, O., Fluorescent retrograde neuronal labeling in rat by means of substances binding specifically to adenine-thymine rich DNA, *Neurosci. Lett.*, 12 (1979) 235–240.
2 Blanks, R. H., Giolli, R. A. and Pham, S. A., Projections of the medial terminal nucleus of the accessory optic system upon pretectal nuclei in the pigmented rat, *Exp. Brain Res.*, 48 (1982) 228–237.
3 Blanks, R. H. I., Precht, W. and Torigoe, Y., Afferent projections to the cerebellar flocculus in the pigmented rat demonstrated by retrograde transport of horseradish peroxidase, *Exp. Brain Res.*, 52 (1983) 293–306.
4 Fallon, J. H., Collateralization of monoamine neurons: mesencephalic dopamine projections to caudate, septum, and frontal cortex, *J. Neurosci.*, 1 (1981) 1361–1368.
5 Fallon, J. H. and Loughlin, S. E., Monoamine innervation of the forebrain: collateralization, *Brain Res. Bull.*, 9 (1982) 295–307.
6 Fallon, J. H. and Moore, R. Y., Catecholamine innervation of the basal forebrain. IV. Topography of the dopamine projections to the basal forebrain and neostriatum, *J. comp. Neurol.*, 180 (1978) 545–580.
7 Giolli, R. A., Blanks, R. H. I. and Torigoe, Y., Pretectal and brainstem projections of the medial terminal nucleus of the accessory optic system of the rabbit and rat as studied using anterograde and retrograde neuronal tracing methods, *J. comp. Neurol.*, (1984a) in press.
8 Giolli, R. A., Braithwaite, J. R. and Streeter, T. T., Golgi study of the nucleus of the transpeduncular tract in the rabbit, *J. comp. Neurol.*, 133 (1968) 309–328.
9 Giolli, R. A., Schmued, L. C., Fallon, J. H. and Loughlin, S. E., A possible retinal-mesotelencephalic pathway in the rat, *Anat. Rec.*, 208 (1984) 61A–62A.
10 Hall, R. D. and Lindholm, E. P., Origin of motor and somatosensory neocortex in the albino rat, *Brain Research*, 66 (1974) 23–38.
11 Hayhow, W. R., Webb, C. and Jervie, A., The accessory optic fiber system in the rat, *J. comp. Neurol.*, 115 (1960) 187–215.
12 Koda, L. Y., Battenberg, E. L. F. and Bloom, F. E., Quantification of neurotransmitter-specific fibers and terminals. In J. E. Johnson, Jr. (Ed.), *Vol. 13*, CRC Press, Boca Raton, FL, 1981, pp. 15–32.
13 Kostovic, I., The terminal distribution of accessory optic fibers in the rat, *Brain Research*, 31 (1971) 202–206.
14 Lenn, N., An electron microscopic study of accessory optic endings in the rat medial terminal nucleus, *Brain Research*, 43 (1972) 622–628.
15 Maekawa, K. and Takeda, T., Electrophysiological identification of the climbing and mossy fiber pathways from the rabbit's retina to the contralateral cerebellar flocculus, *Brain Research*, 109 (1976) 169–174.
16 Maekawa, K. and Takeda, T., Afferent pathways from the visual system to the cerebellar flocculus of the rabbit. In Baker and Berthoz (Eds.), *Control of Gaze by Brain Stem Neurons, Vol. 1*, Elsevier/North-Holland Biomedical Press, Holland, 1977, pp. 187–195.
17 Maekawa, K. and Takeda, T., Origin of descending afferents to the rostral part of dorsal cap of inferior olive which transfers contralateral optic activities to the flocculus. A horseradish peroxidase study, *Brain Research*, 172 (1979) 393–405.
18 Moore, R. Y. and Bloom, F. F., Central catecholamine neuron systems: anatomy and physiology of the dopamine system, *Ann. Rev. Neurosci.*, (1978) 129–169.
19 Pascuzzo, G. J. and Skeen, L. C., Brainstem projections to the frontal eye field in the cat, *Brain Research*, 241 (1982) 341–346.
20 Robertson, R., Efferents of the pretectal complex: separate population of neurons project to lateral thalamus and to inferior olive, *Brain Research*, 258 (1983) 91–95.
21 Schubert, P. and Kreutzberg, G., [^{3}H]Adenosine, a tracer for neuronal connectivity, *Brain Research*, 85 (1975) 317–319.
22 Simon, H., Le Moal, M., Galey, D. and Cardo, B., Silver impregnation of dopaminergic systems after radiofrequency and 6-OHDA lesions of the rat ventral tegmentum, *Brain Research*, 115 (1976) 215–231.
23 Simon, H., LeMoal, M. and Calas, A., Efferents and afferents of the ventral tegmental-A10 region studied after local injection of [^{3}H]leucine and horseradish peroxidase, *Brain Research*, 178 (1979) 17–40.
24 Simpson, J. I., Giolli, R. A. and Blanks, R. H. I., Neuronal connections of the pretectal and accessory optic nuclei to preoculomotor, oculomotor and precerebellar centers of the brain stem. In J. Buttner-Ennever (Ed.), *Progress in Oculomotor Research: Neuroanatomy of the Oculomotor System*, 1984, in press.
25 Simpson, J. I., Soodak, R. and Hess, R., The accessory optic system and its relation to the vestibulocerebellum, *Progr. Brain Res.*, 50 (1978) 715–724.
26 Swanson, L., The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat, *Brain Res. Bull.*, 9 (1982) 321–353.
27 Takeda, T. and Maekawa, K., Bilateral visual inputs to the dorsal cap of inferior olive: differential localization and inhibitory interaction, *Exp. Brain Res.*, 39 (1980) 461–471.
28 Ungerstedt, U., Stereotaxic mapping of the monoamine pathways in the rat brain, *Acta physiol. scand.*, Suppl. 367 (1971).