4. C. M. Jacque, M. L. Harpin, N. A. Baumann, *Eur. J. Biochem.* **11**, 218 (1969); N. A. Baumann, C. M. Jacque, S. A. Pollet, M. L. Harpin, *ibid.* **4**, 340 (1968).
5. S. Pollet, J. M. Bourre, N. A. Baumann, *C. R. H. Acad. Sci. Ser. D* **268**, 2146 (1969).
6. J. M. Bourre, S. Pollet, O. Daudu, N. A. Baumann, *ibid.* **273**, 1534 (1971).
7. ——— in Abstracts, 15th International Conference on the Biochemistry of Lipids, The Hague, Netherlands, 1972, p. 32.
8. J. M. Bourre, S. Pollet, G. Dubois, N. A. Baumann, *C. R. H. Acad. Sci. Ser. D* **271**, 1221 (1970); S. Pollet, J. M. Bourre, O. Daudu, N. A. Baumann, *ibid.* **273**, 1426 (1971); S. Pollet, J. M. Bourre, G. Chaix, O. Daudu, N. A. Baumann, *Biochimie*, in press.
9. The soluble fatty acid synthetase has a molecular weight of 600,000, as estimated by chromatography on Sepharose 4-B. Palmitate is the principal product (93 percent), largely in the unesterified form.
10. Brain tissue contains high levels of enzymes that cleave long-chain acyl-CoA derivatives such as palmitoyl-CoA and stearoyl-CoA. These thioesterases occur both in the cytoplasm [S. Kurooka, K. Hosoki, Y. Yoshimura, *J. Biochem.* **71**, 625 (1972)] and in the microsomal fraction [S. Pollet, J. M. Bourre, O. Daudu, N. Baumann, *C. R. H. Acad. Sci. Ser. D* **273**, 1632 (1971)]. We found that microsomal or cytoplasmic mouse brain thioesterases also hydrolyze arachidyl-CoA. Neither their relative substrate specificities (for $C_{16}$-CoA, $C_{18}$-CoA, or $C_{20}$-CoA) nor their total activities differ significantly in normal and quaking mice. Also, the changes in thioesterase activity (with $C_{16}$-CoA or $C_{18}$-CoA as substrates) in normal mouse brain as a function of age bear no evident relation to the time course of $C_{16}$-CoA and $C_{18}$-CoA elongating activities. It therefore seems unlikely that altered thioesterase levels are responsible for the changed long-chain fatty acid pattern in the brain lipids of the quaking mouse.
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## Dopaminergic Terminals in the Rat Cortex

Abstract. *The destruction of ascending noradrenergic pathways by bilateral microinjections of 6-hydroxydopamine made laterally to the pedunculus cerebellaris superior completely abolished the in vitro synthesis of [$^3$H]norepinephrine from* L-[$^3$H]*tyrosine in slices and in synaptosomes of the rat cortex. However, normal [$^3$H]dopamine synthesis could still be observed in both cortical preparations from animals with lesions. These results provide the first biochemical support for the existence of dopaminergic terminals independent of noradrenergic terminals in the rat cortex.*

Histochemical and lesion studies have indicated three main ascending dopaminergic systems in the rat brain: the nigrostriatal pathway, which is important in extrapyramidal processes (*1*); the tuberoinfundibular system, which is particularly involved in neuroendocrine mechanisms (*2*); and the mesolimbic system (*3*), whose functions are not yet known. High concentrations of dopamine (DA) have been found in the structures innervated by these systems (*4*). Dopamine has also been observed in other brain regions, but it was considered to be present not as a neurotransmitter but as the precursor of norepinephrine (NE). For instance, the cerebellum and the cortex, both innervated by noradrenergic neurons originating from the locus coeruleus, contain not only NE but also DA (*5*). However, DA, which is almost undetectable in the cerebellum by classical spectrofluorimetric techniques, is present in measurable amounts in the cerebral cortex. In this structure we found that DA concentrations were more than 50 percent of those of NE (*6*). This observation led us to investigate whether cortical DA was localized only in noradrenergic terminals in the rat cortex. In an initial study, we observed that electrolytic or chemical destruction of the noradrenergic ascending pathways resulted in 80 to 100 percent reduction in cortical NE levels, whereas those of DA were only slightly affected (*6*). These results revealed the existence of extranoradrenergic stores of DA. We now report not only the occurrence of extranoradrenergic stores of DA, but also the presence of DA terminals in the rat cerebral cortex.

Groups of eight Charles River male rats (300 to 350 g) were killed by decapitation 5 weeks after bilateral chemical lesions made laterally to the pedunculus cerebellaris superior (LPCS) [plane $A_{160}$, König and Klippel atlas (*7*)] to specifically destroy the noradrenergic ascending pathways. Chemical lesions were made while animals were under chloral hydrate anesthesia (500 mg per kilogram of body weight) by microinjection of 6-hydroxydopamine (6-OHDA) (*8*) with the use of a Stoelting stereotaxic apparatus. Sham-operated animals were used as controls.

If DA is found in the cortex after destruction of the noradrenergic ascending pathways, DA synthesis from tyrosine should persist whereas that of NE should be markedly reduced. This was first demonstrated in vitro in cortical slices. Immediately after each animal was killed, the cortex was dissected out and 200 mg of cortical tissues was cut in slices (0.4 mm thick) with a McIllwain apparatus. The slices were incubated in 2 ml of physiological medium (*9*) at 37°C for 15 minutes under a constant stream of 95 percent $O_2$ and 5 percent $CO_2$ in the presence of 68 $\mu$c of L-[3,5-$^3H_2$]tyrosine (40 c/mmole) (Commissariat à l'Énergie Atomique, France). At the end of the incubation period, [$^3$H]DA and [$^3$H]NE in tissues and incubating medium were estimated. Labeled catecholamines were first extracted with ethanol-water (74 : 16) and then separated from $^3$H-labeled precursors and amine metabolites by successive ion exchange chromatography on Amberlite CG 50 and adsorption on alumina as described (*10*). They were finally separated by ion exchange chromatography on Dowex AG 50 WX4 (*11*). Tyrosine was separated by ion exchange chromatography on Dowex AG 50 (H+ form) and estimated spectrofluorimetrically (*12*).

The combined destruction of the ventral and dorsal noradrenergic ascending pathways induced an almost complete disappearance of [$^3$H]NE formation (2.5 percent of control values) (Table 1). This effect was not associated with a parallel decrease in [$^3$H]DA synthesis. The accumulated [$^3$H]DA in slices and incubation medium still represented 46

Table 1. Synthesis of $^3$H-labeled catecholamines from L-[$^3$H]tyrosine in cortical slices and in purified synaptosomal preparations of rats with lesions of noradrenergic ascending pathways made by 6-OHDA microinjection. Protein was estimated by the spectrophotometric method of Lowry *et al.* (*21*). Controls were sham-operated rats.

| Group | Tyrosine ($\mu$g per gram of wet tissue) | [$^3$H]Norepinephrine (nc per gram of wet tissue) | [$^3$H]Dopamine (nc per gram of wet tissue) |
|---|---|---|---|
| | *Cortical slices* | | |
| Control | 15.9 ± 1.6 | 171.8 ± 20.0 | 152.2 ± 13.1 |
| 6-OHDA | 14.5 ± 0.4 | 4.4 ± 0.7* | 70.1 ± 10.0* |
| | *Cortical synaptosomes* | | |
| Control | 0.210 ± 0.006 | 5.250 ± 0.200 | 8.85 ± 0.28 |
| 6-OHDA | 0.189 ± 0.005 | 0.340 ± 0.002* | 5.78 ± 0.25* |

* $P < .001$, Student's $t$-test.

percent of the labeled amines synthesized in sham-operated rats. The degeneration of noradrenergic terminals did not induce appreciable changes in the concentration of endogenous tyrosine, since the amounts of this amino acid in the cortex of rats with lesions and sham-operated controls were not statistically different (Table 1).

In a separate experiment, we further characterized the $^3$H-labeled amines synthesized from L-[$^3$H]tyrosine (68 $\mu$c) in cortical slices of rats with lesions and controls. Labeled catecholamines were eluted from the alumina columns and acetylated, and the labeled acetylamine derivatives were separated by paper chromatography (*13*). As indicated in Fig. 1, [$^3$H]NE was no longer detectable in cortical extracts from rats with 6-OHDA lesions; however, [$^3$H]DA was still formed in great quantity from L-[$^3$H]tyrosine (*14*).

To determine whether the [$^3$H]DA detectable in cortical slices of rats with 6-OHDA lesions was synthesized extraneuronally or in nerve terminals, the synthesis of $^3$H-labeled catecholamines was estimated on a purified synaptosomal preparation. Cortices of five rats with lesions and those from five sham-operated rats were homogenized in 0.32*M* sucrose, and synaptosomes (B band) were separated after differential centrifugation in discontinuous sucrose gradients (*15*). The synaptosomal preparation obtained from each group of rats was resuspended in 8.5 ml of physiological medium (*9*) and divided into eight 1-ml samples which were then incubated separately at 37°C for 15 minutes under 95 percent $O_2$ and 5 percent $CO_2$ in the presence of 53 $\mu$c of L-[$^3$H]tyrosine. Total $^3$H-labeled catecholamines accumulated both in tissues and incubating medium were extracted and separated as described earlier. Results were similar to those for cortical slices (Table 1). Synthesis of [$^3$H]NE was reduced to less than 10 percent in synaptosomes from rats with 6-OHDA lesions. On the contrary, [$^3$H]DA synthesis was only slightly reduced, to 65 percent of that in synaptosomes from sham-operated animals. As in cortical slices, no statistical difference could be seen between the endogenous levels of tyrosine in synaptosomal preparations from rats with lesions or controls (Table 1).

As previously reported, microinjections of 6-OHDA in the LPCS area induce a complete disappearance of noradrenergic terminals in the rat cortex (*6*). The complete suppression of [$^3$H]-NE synthesis from [$^3$H]tyrosine in cortical slices and in synaptosomes is in agreement with the 90 percent reduction of NE levels observed 5 weeks after LPCS lesions (*6*). These results further support the anatomical mapping of noradrenergic ascending pathways by histochemical methods (*3*).

On the other hand, we have reported that DA levels in the rat cortex were only slightly affected after the destruction of cortical noradrenergic terminals (*6*). The persistence of [$^3$H]DA synthesis from L-[$^3$H]tyrosine in cortical slices and synaptosomes of rats with LPCS lesions (Table 1) is in agreement with the previous observation. However, [$^3$H]DA synthesis in rats with lesions was reduced 40 to 54 percent as compared with that in sham-operated animals. This may be attributed to the degeneration of noradrenergic pathways and consequently to the lack of formation of [$^3$H]DA, the precursor of [$^3$H]-NE in these terminals. Nevertheless, a change in [$^3$H]DA synthesis induced by the absence of noradrenergic terminals in the cortex may be partly involved in this effect.

The capacity of cortical synaptosomes

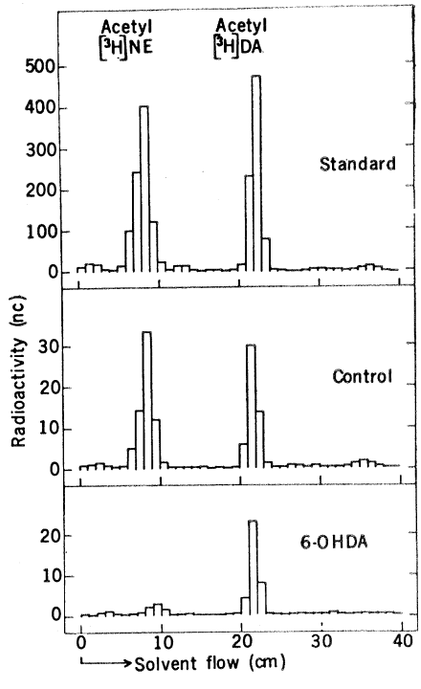


Fig. 1. Chromatography of acetyl derivatives of $^3$H-labeled catecholamine synthesized from L-[$^3$H]tyrosine in cortical slices of rats with 6-OHDA lesions and sham-operated controls. The standard shows chromatography of acetyl derivatives of [$^3$H]DA and [$^3$H]NE previously purified and treated as tissue samples. Acetylation was done on pooled samples obtained from two alumina eluates.

to continue to synthesize [$^3$H]DA from L-[$^3$H]tyrosine after degeneration of noradrenergic terminals strongly suggests the existence of dopaminergic terminals in the rat cortex. Synthesis of [$^3$H]DA has also been observed in rat cortical synaptosomes after electrolytic lesions of the locus coeruleus, lesions that induce a complete degeneration of the dorsal noradrenergic pathway (*16*). A sprouting of dopaminergic terminals originating from the mesolimbic or nigrostriatal dopaminergic systems could also explain the DA synthesis observed in rats with lesions of ascending noradrenergic neurons. However, this seems unlikely, since endogenous levels of DA are similar in rats with lesions and sham-operated controls (0.140 ± 0.029 $\mu$g/g and 0.122 ± 0.007 $\mu$g/g, respectively). Furthermore, synthesis of [$^3$H]-DA from L-[$^3$H]tyrosine was also found in cortical synaptosomes 14 days after destruction of noradrenergic pathways, when sprouting is not thought to occur. Thus there is evidence that the rat cortex contains not only noradrenergic but also dopaminergic terminals.

The localization of the cell bodies of these dopaminergic neurons remains to be established. Until now, we have failed to see marked changes in cortical DA levels after lesions of the medial forebrain bundle in the lateral hypothalamus and of the $A_{10}$ area which induced degeneration of the mesolimbic system. Although no data are available, it cannot be excluded that the rat cortex contains dopaminergic interneurons. In fact, the existence of cortical cell bodies that accumulate higher concentrations of exogenous NE has been shown in histochemical (*17*) as well as radioautographic (*18*) studies. A similar situation may also hold for the mammalian cortex, inasmuch as finite amounts of homovanillic acid have been measured in the cortex of other species (*4, 19*). Cortical DA terminals may be important in the pharmacological effects of psychotropic drugs that affect central DA metabolism, such as persistence of many amphetamine actions after intraventricular injection of 6-OHDA (*20*).

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**References and Notes**

1. N. E. Anden, A. Carlsson, A. Dahlström, K. Fuxe, N. A. Hillarp, K. Larsson, *Life Sci.* **3**, 523 (1964); A. Hornykeiwicz, *Pharmacol. Rev.* **18**, 925 (1966).
2. K. Fuxe and T. Hökfelt, *Acta Physiol. Scand.* **66**, 245 (1966); J. Barry, *C. R. Seances Soc. Biol.* **162**, 1946 (1968); C. Kordon and J. Glowinski, *Endocrinology* **85**, 924 (1966); K. Fuxe and T. Hökfelt, *Aspects of Neuroendocrinology* (Springer-Verlag, Berlin, 1970), pp. 192–205.
3. N. E. Anden, A. Dahlström, K. Fuxe, K. Larsson, L. Olson, U. Ungerstedt, *Acta Physiol. Scand.* **67**, 313 (1966); U. Ungerstedt, *Acta Physiol. Scand. Suppl. 367* (1971).
4. A. Bertler and E. Rosengren, *Acta Physiol. Scand.* **47**, 350 (1959); R. Laverty and D. F. Sharman, *Brit. J. Pharmacol.* **24**, 538 (1965).
5. S. H. Koslow, F. Cattabeni, E. Costa, *Science* **176**, 177 (1972); L. Valzelli and S. Garattini, *J. Neurochem.* **15**, 259 (1968).
6. A. M. Thierry, L. Stinus, G. Blanc, J. Glowinski, *Brain Res.* **50**, 230 (1973). The DA and NE contents of the cerebral cortex were $0.122 \pm 0.007$ μg/g and $0.213 \pm 0.021$ μg/g, respectively.
7. J. F. R. König and R. A. Klippel, *The Rat Brain* (Williams & Wilkins, Baltimore, 1963).
8. 6-Hydroxydopamine (2 μg) in 1 μl of an isotonic saline solution containing ascorbic acid (2 mg/ml) adjusted at *p*H 4 was delivered at a rate of 1 μl/5 min [Y. Agid, F. Javoy, C. Sotelo, D. Bouvet, J. Glowinski, *Brain Res.* **58**, 291 (1973)]. The coordinates are P, 6 mm posterior to the bregma; L, 1.4 mm lateral from the midline; and H, 7.7 mm below the calvarium.
9. The physiological medium contained 5 m*M* glucose, 136 m*M* NaCl, 5.6 m*M* KCl, 1.2 m*M* $MgCl_2$, 2.2 m*M* $CaCl_2$, and 16.2 m*M* $NaHCO_3$.
10. A. M. Thierry, G. Blanc, J. Glowinski, *J. Neurochem.* **18**, 449 (1971).
11. J. Glowinski, L. L. Iversen, J. Axelrod, *J. Pharmacol. Exp. Ther.* **151**, 385 (1966).
12. S. Udenfriend, *Fluorescence Assay in Biology and Medicine* (Academic Press, New York, 1962), vol. 1, pp. 125–169.
13. R. Laverty and D. F. Sharman, *Brit. J. Pharmacol.* **24**, 538 (1963).
14. In initial experiments, persistent synthesis of [$^3$H]DA from L-[$^3$H]tyrosine could also be demonstrated in vivo after intracisternal injection of the labeled amino acid in rats whose ascending noradrenergic systems had been similarly destroyed by microinjection of 6-OHDA in the LPCS area.
15. E. G. Gray and V. P. Whittaker, *J. Anat.* **96**, 79 (1963); M. Israel and P. Frachon-Mastour, *Arch. Anat. Microscop. Morphol. Exp.* **59**, 383 (1970).
16. A. M. Thierry, G. Blanc, A. Sobel, L. Stinus, J. Glowinski, unpublished observations.
17. G. W. Arbuthnott, *J. Neurochem.* **16**, 1599 (1969).
18. L. Descarries and Y. Lapierre, *Brain Res.* **51**, 141 (1973).
19. P. L. McGeer, J. A. Wada, E. G. McGeer, *Recent Advan. Biol. Psychiat.* **5**, 228 (1963); P. Mathieu, J. C. Charvet, G. Chazot, P. Trouillas, *Clin. Chim. Acta* **41**, 5 (1972).
20. K. D. Evetts, N. J. Uretsky, L. L. Iversen, S. D. Iversen, *Nature* **225**, 961 (1970).
21. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).


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## Alkylpyrazine Alarm Pheromones in Ponerine Ants


Abstract. *The mandibular gland secretions of the ponerine ants* Odontomachus hastatus, O. clarus, *and* O. brunneus *contain alkylpyrazines. These compounds, 2,5-dimethyl-3-isopentylpyrazine in* O. hastatus *and* O. clarus, *and 2,6-dimethyl-3-pentyl-, -butyl-, -propyl-, and -ethyl-pyrazines in* O. brunneus, *have previously not been found as animal natural products. These compounds function as powerful releasers of alarm behavior for* Odontomachus *workers and are probably also utilized as defensive compounds.*


The mandibular glands of ants, particularly species in the subfamilies Myrmicinae and Formicinae, have proved to be an outstanding source of natural products which function as releasers of behavior (*1*). Aliphatic carbonyl compounds are characteristically produced in these exocrine structures (*1*) and are often employed as powerful alarm pheromones. Similar behavior is displayed by many species in the primitive subfamily Ponerinae, but the mandibular gland products liberated by ponerine species are generally identified with distinctive odors which we have not encountered in other subfamilies of ants.

Disturbed worker ants of the large neotropical species *Odontomachus hastatus* (*2*), as well as other ponerine species, discharge a secretion having the characteristic odor of chocolate. Similarly, the mandibular gland secretions of *O. brunneus* and *O. clarus* (*2*), two remarkably protean species in this subfamily (*3*), also possess this aroma. The mandibular gland secretions (*4*) of these three species of *Odontomachus* contain various alkylpyrazines, none of which have been previously identified as exocrine products of animals. Significantly, these compounds possess the chocolate odors that are identified with the glandular exudates.

Methylene chloride or *n*-pentane extracts of *O. hastatus* and *O. clarus* heads were analyzed by combined gas chromatography and mass spectrometry (*5*). The major volatile component exhibited a base peak at *m/e* (mass/charge) 122 and additional peaks at *m/e* 177, 163, 149, 135, 80, and 42. Similar examination of extracts of *O. brunneus* heads indicated an apparent isomer of the compound from *O. hastatus*. Although both isomers showed a base peak at *m/e* 122, they could be distinguished by the relative intensities of the peaks at *m/e* 177, 163, 149, and 135. Of more significance, the isomer from *O. brunneus* exhibited a molecular ion at *m/e* 178 which was conspicuously absent in the other isomer. The second extract had, in addition, three lower homologs with molecular weights of 164, 150, and 136. The first two of these also exhibited a base peak at *m/e* 122.

A computer search of mass spectra (*6*) indicated that 2,5-dimethyl-3-isopentylpyrazine (**1**) has a fragmentation pattern very similar to that of the *O. hastatus* volatile. Although only 2,5-dimethyl-3-alkylpyrazines (**1**, **4**, and **5**) have been identified as major components of cocoa aroma (*7*), 2,6-dimethyl-3-alkylpyrazines give essentially identical mass spectra (*8*). Therefore, each of the alkylpyrazines (**1** to **12**) was synthesized from either 2,5- or 2,6-dimethylpyrazine (*9*) and the appropriate alkyl lithium (*10*). The mass spectra and retention times of **7**, **9**, **10**, and **11** were identical to those of the compounds present in *O. brunneus* extracts, and the mass spectrum and retention time of **1** were identical to that of the compound present in *O. hastatus* and *O. clarus*. *Odontomachus clarus* also contained a small amount of **5**.

Although the mass spectra of the 2,6-dimethyl-3-alkylpyrazines are essentially identical to those of the 2,5-isomers, the retention times of the 2,5-isomers are significantly shorter than the 2,6-isomers under isothermal conditions. Further confirmation of the occurrence of the 2,5-isomer in *O. hastatus* and *O. clarus* and the 2,6-isomers in *O. brunneus* was obtained by quaternization of the pyrazines with methyl iodide. The quaternized products were reduced with sodium borohydride to piperazines. The monomethylated piperazines from *O. hastatus* and *O. clarus* and from 2,5-dimethyl-3-isopentylpyrazine exhibited a base peak at *m/e* 72 and were identical. On the other hand, the monomethylated piperazines from *O. brunneus* and from 2,6-dimethyl-3-*n*-pentylpyrazine exhibited a base peak at *m/e* 128 and were identical. Similar treatment of other isomers showed that this difference is attributable to the ring substitution rather than differences in the side chain.

Pyrazine **1** constitutes 77 percent of the *O. hastatus* secretion, and pyrazines **7**, **9**, **10**, and **11** are present in *O. brunneus* extracts in 91, 7, 1.4, and 0.6 percent, respectively. An unidentified species of *Odontomachus* from Puerto Viejo, Costa Rica, contains 2,6-dimethyl-3-*n*-pentylpyrazine as the major component along with small amounts