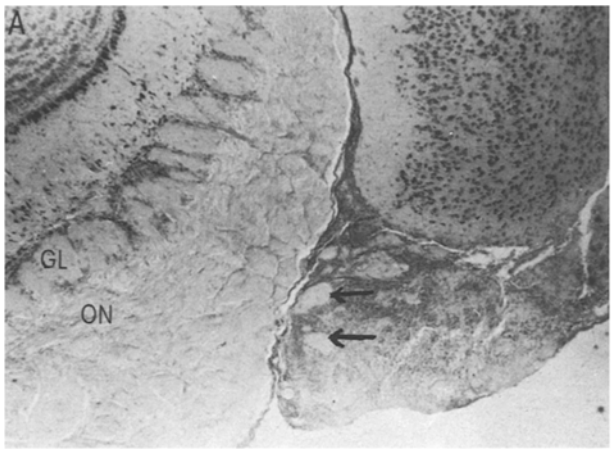
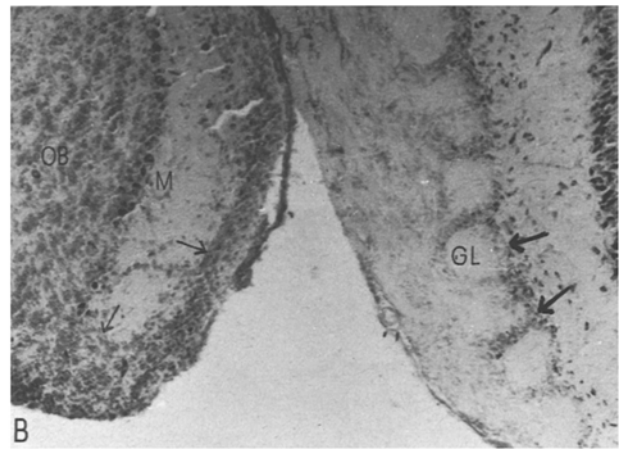


A On the right, the regenerated nerve has formed glomerulus-like structures (arrows) underneath the protruded cortex in the absence of the olfactory bulb and retrobulbar structures. On the left, the intact olfactory (OB) bulb with normal glomeruli (GL) and the plexus formed by the olfactory nerve (ON) can be seen.



B Coronal section through the olfactory bulb. On the right, the control bulb (OB) is seen with glomeruli GL surrounded by periglomerular cells (arrows). On the left, a portion of the olfactory bulb (OB) which was spared from the lesion is seen to contain mitral (M) and periglomerular cells (arrows), but the glomeruli have not been formed.

ventral side of the protruded cortex, contained glomerulus-like structures (figure A). Inspection of serial sections revealed that the cells surrounding these structures formed a cell stream along the known migratory path of the postnatal bulbar cells, reaching the subependymal layer of the lateral ventricle. In 1 brain, glomerulus-like structures were seen to penetrate the brain along this path. In a few brains, however, the plexus formed by the ON was surrounded by cells without forming any glomerulus-like structures. In specimens where a portion of the anterior olfactory nucleus was spared, these glomerulus-like structures were seen to lie within the peripheral parts of this structure. In cases where a portion of the OB was spared but no indication of ON regeneration could be discerned by the light microscope, mitral and periglomerular cells occupied their appropriate position without forming any of these glomerulus-like structures (figure B). Although similar to the GL, these glomerulus-like structures form round bodies within the plexus of the ON and are surrounded by cells which appear to have the same origin as the PGLC, unlike the GL they lack the dendritic contribution from mitral and tufted cells, besides the synaptic organization within them is, as yet, unknown. Therefore, we prefer to call them glomerulus-like structures rather than refering to them as proper glumeruli.

In summary, findings described here show that glomerulus-like structures can be formed in the abscence of mitral and tufted cells as long as the regenerated ON is present. On the other hand, these structures do not form in the absence of the ON, even when mitral and tufted cells are present. Thus, although no definite conclusion can be reached about the mechanism of glomerular formation during normal growth, findings presented here indicate that formation of these postnatal structures are induced by the ON and are independent of mitral and tufted cells. Further, since the regenerated ON can form structures in the absence of its natural target, i.e., the OB, this system can serve as an interesting mammalian model for the study of neural plasticity and factors that guide the axons to their targets.

1 I am grateful to Miss Sekineh Hamedi and Mr Davood Ahmadi for their assistance and to Dr Esmail Meisami for critical discussion of the topic. Reprint requests should be addressed to: 140 Flora Avenue, Apt. 133, Walnut Creek (California 94595, USA).

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Topographical distribution of ATP in rat brain

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Summary. Studies on the distribution of ATP in microdissected segments of the rat brain indicate that the nucleotide is concentrated in gray matter, and especially in the thalamus, hypothalamus, hippocampus, entorhinal cortex and sensorimotor cortex. These distribution studies in conjunction with previous neuropharmacological studies, support the concept that adenine nucleotides may function as intercellular mediators in various regions of the brain.

Adenosine triphosphate (ATP) has been proposed as a candidate for a neurotransmitter role in the peripheral nervous system[1], raising the possibility that it may also be a transmitter in the central nervous system (CNS). Previous reports[2-4] have described a powerful depressant action of adenine derivatives on the discharges of cerebral and cerebellar cortical neurones. This effect appears to result from the activation of an extracellularly located purine

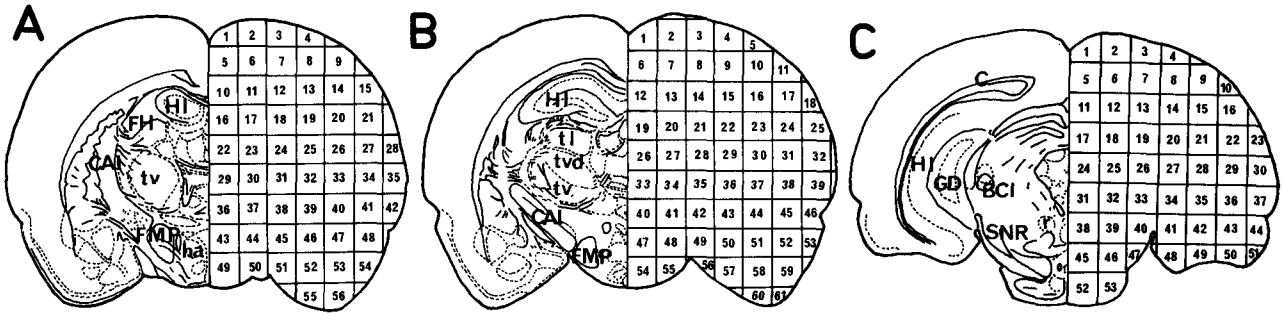
receptor[5]. Amongst the various brain regions tested, neurones in the hippocampus were most sensitive to iontophoretically applied adenine nucleotides[6].

Previous reports have described the presence of ATP in the mammalian CNS[7-10]. These studies, together with the evidence that ATP can be released from rat cortex[7] and synaptosomal preparations[11] strongly suggest that ATP or other adenine derivatives may participate in central neuronal transmission[5,12]. However, knowledge concerning the distribution of potential purinergic pathways in the central nervous system is presently very limited, as the reliable identification of purinergic pathways by histochemical or immunochemical methods is not yet possible. In order to gain some insight into the distribution of purinergic pathways in rat brain, we have measured the ATP concentration

ATP content of microdissected brain slices

5150 µm (A)* Segments	ATP (nmoles/segment)	3990 µm (B)* Segments	ATP (nmoles/segment)	1270 µm (C)* Segments	ATP (nmoles/segment)
1	0.27±0.16	1	0.60±0.29	1	0.32±0.2
2	0.28±0.17	2	0.37±0.14	2	0.44±0.12
3	0.27±0.18	3	0.30±0.22	3	0.25±0.13
4	0.31±0.21	4	0.50±0.20	4	0.41±0.16
5	0.25±0.13	5	0.32±0.13	5	0.31±0.06
6	0.16±0.09	6	0.33±0.15	6	0.20±0.06
7	0.10±0.07	7	0.27±0.12	7	0.23±0.07
8	0.19±0.14	8	0.56±0.71	8	0.25±0.10
9	0.15±0.21	9	0.47±0.56	9	0.39±0.13
10	0.12±0.01	10	0.36±0.23	10	-
11	0.19±0.09	11	0.43±0.14	11	0.31±0.11
12	0.29±0.20	12	0.29±0.15	12	0.29±0.07
13	0.25±0.16	13	0.32±0.16	13	0.26±0.14
14	0.24±0.17	14	0.23±0.12	14	0.29±0.08
15	0.25±0.21	15	0.31±0.15	15	0.48±0.31
16	0.25±0.17	16	0.21±0.10	16	0.44±0.17
17	0.22±0.08	17	0.27±0.14	17	0.27±0.06
18	0.30±0.19	18	0.39±(0)	18	0.28±0.12
19	0.23±0.20	19	0.39±0.15	19	0.21±0.09
20	0.24±0.13	20	0.42±0.33	20	0.38±0.14
21	0.18±0.08	21	0.41±0.21	21	0.37±0.20
22	0.31±0.10	22	0.52±0.21	22	0.43±0.23
23	0.26±0.12	23	0.41±0.19	23	0.32±0
24	0.31±0.15	24	0.26±0.15	24	0.28±0.09
25	0.28±0.22	25	0.32±0.3	25	0.28±0.14
26	0.18±0.10	26	0.50±0.24	26	0.30±0.12
27	0.23±0.19	27	0.35±0.16	27	0.34±0.11
28	0	28	0.25±0.29	28	0.32±0.15
29	0.29±0.12	29	0.31±0.26	29	0.30±0.16
30	0.44±0.29	30	0.43±0.39	30	0.45±(0)
31	0.35±0.21	31	0.62±0.77	31	0.30±0.17
32	0.32±0.17	32	0.28±0.24	32	0.20±0.08
33	0.24±0.21	33	0.37±0.16	33	0.39±0.16
34	0.38±0.31	34	0.38±0.20	34	0.33±0.22
35	0	35	0.43±0.32	35	0.46±0.39
36	0.28±0.16	36	0.32±0.18	36	0.43±0.33
37	0.43±0.28	37	0.30±0.17	37	0.26±0
38	0.39±0.20	38	0.31±0.23	38	0.50±0.27
39	0.32±0.14	39	0.73±(0)	39	0.29±0.23
40	0.17±0.15	40	0.38±0.27	40	0.28±0.07
41	0.30±0.23	41	0.30±0.19	41	0.21±0.14
42	0	42	0.29±0.17	42	0.32±0.16
43	0.52±0.3	43	0.26±0.22	43	0.41±0
44	0.26±0.14	44	0.33±0.06	44	-
45	0.29±0.21	45	0.39±0.13	45	0.60±0.31
46	0.26±0.21	46	-	46	0.36±0.17
47	0.27±0.23	47	0.41±0.14	47	0.40±0.20
48	0.64±0.75	48	0.41±0.23	48	0.47±0.29
49	0.34±0.14	49	0.24±0.10	49	0.36±0.23
50	0.36±0.11	50	0.30±0.17	50	-
51	0.38±0.15	51	0.37±0.16	51	-
52	0.31±0.19	52	0.61±0.05	52	-
53	0.52±0.53	53	0	53	-
54	0.13±0	54	0.17±0.11	54	-
55	-	55	0.28±0		
56	-	56	0.11±(0)		
		57	0.29±0.22		
		58	0.70±0.6		
		59	0.78±0		
		60	-		

Values are presented as mean ± SD of 5 experiments. *See figure A, B and C for localization of numbered segments.



Each rat brain was fixed in a Gerling-Moore Metabostat microwave unit (Palo Alto, Calif.) at 3.6 KW, 2.0 sec. The brain was removed and frozen to −8 °C immediately after irradiation. Coronal sections (300-µm thickness) of brain were cut on a cryostat. The sections were mounted on glass slides and allowed to dry 2 h at room temperature. The sections taken at A 5150 µm, B 3990 µm, C 1270 µm according to the atlas of Konig and Kippel were further divided into 1 mm^2 segments. Each segment was labelled and homogenized in 200 µl of 200 mM Tris-HCl Buffer. ATP concentrations were determined according to the method described in the text. Abbreviations. *A* HI: hippocampus, FH: fimbria hippocampus, CAI: internal capsule, tv: ventral thalamic nucleus, ha: anterior hypothalamic nucleus, FMP: medial forebrain bundle. *B* HI: hippocampus, tl: lateral thalamic nucleus, tvd: central dorsal thalamic nucleus, tv: ventral thalamic nucleus, CAI: internal capsule, FMP: medial forebrain bundle. *C* HI: hippocampus, GD: dentate gyrus, r: red nucleus, C: cingulum, BCI: brachium of inferior colliculus, SNR: substantia nigra.

in a series of brain segments and used this data to construct a map of its distribution in the brain.

Methods. Sprague Dawley rats (200–300 g) were subjected to microwave irradiation (2.0 sec) at 3.6 kW. Brains were removed and frozen to −8 °C immediately. Coronal sections (300 µm thick) were made on a cryostat. The 3 sections corresponding to 5150 µm, 3990 µm and 1270 µm of the atlas of Konig and Kippel[13] were dried on glass slides and then further divided into 1 mm × 1 mm squares under a dissecting microscope, using a microscalpel. Each segment (1 mm × 1 mm × 0.3 mm) was homogenized in 200 µl of 200 mM Tris-HCl buffer pH 7.4 containing 70 mM KCl and 12 mM $MgCl_2$. After centrifugation to remove protein, the supernatant was analyzed for ATP by Kimmich's method[14]. 50 mg of soluble extract from dried firefly lanterns (Sigma) was dissolved in 5 ml dist. H_2O immediately before use. To 1 ml of this solution, 0.5 ml arsenate buffer (50 mM, pH 7.4) and 1 ml of Tris-HCl buffer (200 mM, pH 7.4) were added. 250 µl of this enzyme reaction mixture was transferred to a vial (New England Nuclear, Boston) and kept in a light shielded ice box until use. 20 µl of the deproteinized supernatant was added to the above mentioned enzyme reaction mixture, and mixed very rapidly. Counting began precisely 20 sec after adding the supernatant and terminated precisely 20 sec later. An ISOCAP 300 Nuclear Chicago Scintillation spectrophotometer was used for the experiment.

Results and discussion. The rationale for using rat brain coronal section 5150 µm, 3990 µm and 1270 µm for analysis of ATP distribution was to use brain sections containing a maximal number of nuclei in order to obtain a general view of the distribution of this nucleotide in rat brain. It was found that in the section 5150 µm (corresponding to the figure, A), ATP concentrations ranging from 0.35–0.49 nmoles/segment were found in thalamic (figure, A, 24, 25, 31, 32, 37) and hypothalamic nuclei (figure, A, 43, 44, 49, 50). A higher ATP concentration (> 0.49 nmoles/segment) was found in the anterior hypothalamic nuclei (figure, A, 43) and entorhinal cortex (figure, A, 52, 53). A similar ATP distribution was found in the next section at 3990 µm (figure, B). Higher concentrations of ATP were associated with gray rather than with white matter and especially with the various thalamic and hypothalamic nuclei. Sensorimotor cortex (figure, B, 1, 2, 3, 4, 5), entorhinal cortex (figure, B, 52, 59, 45) and hippocampus (figure, B, 15, 16, 17, 22, 23, 24), exhibit the highest ATP concentration amongst the segments examined. When ATP concentration was determined at the level of section 1270 µm (figure, C), it was found that the red nucleus (figure, C, 38) and hippocampus (figure, C, 15, 21, 22, 28, 35, 36) contained higher concentrations of ATP than the other structures in the same section. These results are consistent with the findings of previous investigators[15] that the ATP concentration is higher in gray matter than in white matter. It is especially concentrated in the various thalamic and hypothalamic nuclei and in the hippocampal region.

The recent study on the topographical distribution of ATP in conjunction with reports on the release of adenine nucleotide suggests the potential importance of ATP in CNS transmission. The hippocampus may provide a particularly suitable target for further studies on purinergic transmission. This structure exhibits high concentrations of ATP and contains neurones that are very sensitive to the depressant action of purine nucleotides[6]. Furthermore, Schubert and his colleagues[16] have shown that adenine nucleotides can be released from the molecular layer of the hippocampus by electrical stimulation of an identifiable tract, the perforant pathway.

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