ACTA STEREOL 1985; 4/2: 171—174
PROC ESS IV-1 GÖTEBORG 1985
ORIGINAL SCIENTIFIC PAPER

ON THE USE OF AUTOMATIC IMAGE ANALYSIS IN THE DEMONSTRATION OF TRANSMITTER IDENTIFIED NERVE CELL CLUSTERS IN THE CENTRAL NERVOUS SYSTEM

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ABSTRACT

By means of the "close" function of the IBAS image analyzer it has been possible to determine if the transmitter-identified nerve cell bodies in an area are scattered or form clusters, since closely packed nerve cell bodies will unite by this procedure. The cell density of the cluster can be evaluated by considering the ratio between the area of its nerve cell profiles and the field area of the entire cluster. By this procedure a cluster of adrenaline nerve cell bodies have been discovered in the ventrolateral rostral reticular formation of the medulla oblongata belonging to the lateral part of adrenaline group C1.

Key-words: Brain, clusters, image analysis, nerve cell bodies, nerve cell clusters.

INTRODUCTION

Previously we have been able to describe groups and subgroups of transmitter identified nerve cell populations by means of semi-automatic image analysis in combination with the indirect immunoperoxidase procedure (Agnati et al., 1982, 1984). However, this procedure was found to be time consuming and we have therefore developed methods which involve the use of automatic image analysis to obtain a complementary, but less time consuming procedure for the objective demonstration of clusters of transmitter identified nerve cell bodies in the central nervous system.

THE PRESENT METHOD TO DEMONSTRATE NERVE CELL CLUSTERS BY MEANS OF AUTOMATIC IMAGE ANALYSIS

Firstly, the image is improved by the use of a shading function. In this procedure, usually a background image is used, which has been obtained by the introduction of a grey filter into the pathway of the transmitted light. Thereafter, the perimeter of the transmitter identified cell group is interactively outlined in the editor function. In this example the transmitter identified nerve cell group analyzed is the phenylethanolamine-N-methyl transferase (PNMT) immunoreactive nerve cell group in the ventrolateral and rostral part of the reticular formation of the medulla oblongata (C1 group; Hökfelt et al. 1974). The measurements have been performed on a binary image of this cell group, obtained by the use of an

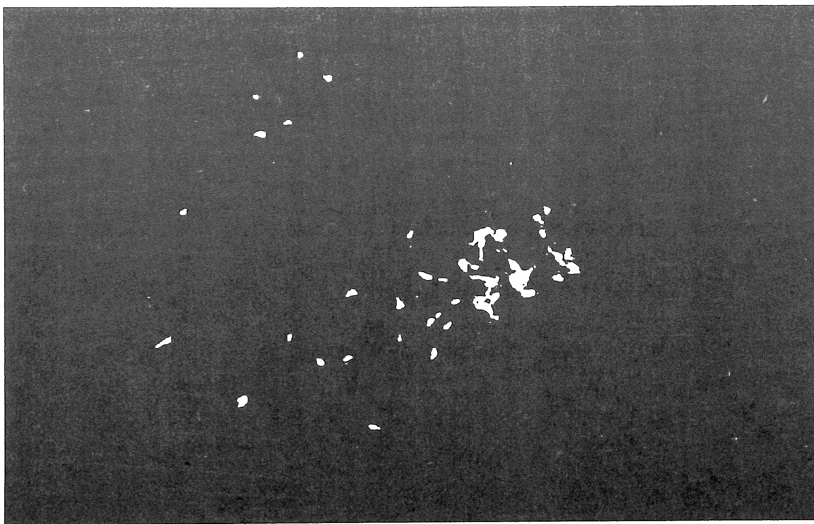

Fig. 1. A binary image showing discrimination of PNMT cell bodies of group C1. $\bar{A}$= 209±138 μm^2, means ± S.D.

interactive discrimination function (Fig. 1). To produce the binary image we used the grey tone level which resulted in the selective demonstration of the PNMT immunoreactive nerve cell bodies. Grey tone levels below this critical value (high optical density) were considered as white, while grey tone levels above this level (low optical density) were considered as black. By means of the identification and measuring programs the area within the perimeter, i.e., the cell group area itself, was measured (A_T). Subsequently, also the mean area ($\bar{A}$) and the number of PNMT immunoreactive nerve cell profiles were measured within the A_T region. These two values were automatically obtained from the histogram of the areas of the PNMT immunoreactive profiles. By means of a "skip" function objects too small or too large to represent nerve cell bodies were automatically removed. By multiplying the number (N) of PNMT immunoreactive cell bodies with the mean area ($\bar{A}$) of these cells the field area (FA_C) of the PNMT immunoreactive nerve cell group could be obtained. The ratio between the field area (FA_C) and the area enclosed by the perimeter of the cell group (A_T) gives an evaluation of the volume fraction of the transmitter identified nerve cell group. To help remove the small immunoreactive profiles in the binary image also a medianization function was included in the program after the discrimination procedure.

The possible existence of a cluster of transmitter identified nerve cell bodies within the area analyzed (A_T) was tested by use of the "close" function (Fig. 2 and Table I) (see Agnati et al., 1985a,b). In this procedure densely packed objects unite to form large individual objects. Formation of such large profiles will give some objective evidence for the existence of clusters in the transmitter identified nerve cell group. Nerve cells far apart will in contrast not form clusters, giving evidence that these in fact are scattered. In the present procedure nerve cell bodies lying less than or 65 µm apart were joined to form clusters. The

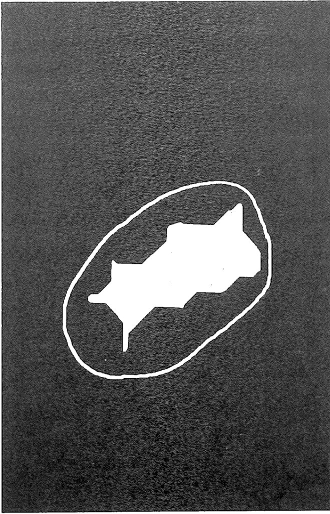

Fig. 2.

Fig. 3.

Fig. 2. By means of the close function a cell cluster can be detected in the image of Fig. 1. The area of the cell cluster has been encircled by means of an interactive procedure. The other PNMT immunoreactive profiles have been removed. The value of the cell group area can be evaluated (A_{C1}) (38080 μm^2).

Fig. 3. Demonstration of the overlap area between images shown in fig. 1 and fig. 2 using the logical operation "and" (Boolop function). In this way the PNMT immunoreactive cell bodies forming the cluster in fig. 2 are shown. $\bar{A}$= 327$\pm$306 μm^2 (means $\pm$ S.D.).

form factor and the area of the clusters formed were then determined interactively in the editor function. By means of the "Boolop" function (logical function "and") the image containing the cluster was compared with the original discriminated image (binary), which allows the determination of the overlap area in the cluster. The overlap region demonstrated in this way represents the field area of the PNMT immunoreactive nerve cell bodies forming the cluster when the close function is triggered (FA_{C1}) (Fig. 3). The ratio between the field area of the cluster (FA_{C1}) and the total area of the cluster (A_{C1}) will give an evaluation of the cell density in the cluster. By dividing the field area of the cluster (FA_{C1}) with the mean area of the individual nerve cell body within the cluster ($\bar{A}_{C1}$) the number of PNMT immunoreactive cell bodies within the cluster can be obtained. If the number was equal or larger than 5 the cluster was accepted. By knowing the total number of nerve cell bodies within the transmitter identified nerve cell population (A_T) it became possible to determine the per cent of nerve cell bodies which appeared scattered within the transmitter identified nerve cell group. In coronal sections the main cluster was formed in the lateral part of the adrenaline nerve cell group C1, located in the rostral part of the ventro-lateral reticular formation of the medulla oblongata of the rat.

TABLE 1 DEMONSTRATION OF PNMT IMMUNOREACTIVE CELL GROUPS OF THE MALE RAT IN CORONAL SECTIONS BY IMAGE ANALYSIS

By means of the IBAS Image Analyzer (see Figs. 4-6, and text), using the "close" function, PNMT immunoreactive cell bodies lying within a distance of 65 μm from each other have been united to form clusters. The five largest clusters found at each rostrocaudal level analyzed have been selected and analyzed. Clusters have been accepted when $FA_{C1}/\bar{A}_{C1} \geq 5$. The $FA_{C1}/\bar{A}_{C1}$ value has been rounded off to the nearest integer value. Gravity Centers (GCx and GCy) are determined by using an Apple IIe computer.

SECTION	CELL BODY PARAMETERS				CELL GROUP PARAMETERS					
Rostro-caudal level (mm rostral (+) or caudal (-) to obex)	Mean area ($\bar{A}$) μm^2	Total counts ($N;A_T$)	Area (A_{C1}) μm^2	Shape Factor (SF) 0 to 1	X-angle (degr)	Field Area (FA_{C1}) μm^2	Gravity Centers GCx μm	GCy μm	FA_{C1}/A_{C1}	$FA_{C1}/\bar{A}_{C1}$*
1) +1.7 mm	537	61								
cluster I accepted (lateral C1)			18200	0.339	131	5631	1953	661	0.309	11
-"- II accepted (medial C1)			6404	0.191	159	3045	1242	644	0.475	6
-"- III accepted (lateral C2)			9546	0.278	148	3045	1036	2436	0.319	6
-"- IV not accepted (medial C2)			4302	0.179	111	1861			0.433	3
-"- V not accepted (medial C1 area)			3673	0.157	63	1668			0.454	3
2) +1.5 mm	421	42								
cluster I accepted (lateral C1)			14770	0.348	139	6211	1880	652	0.421	15
-"- III not accepted (lateral C2)			2489	0.159	27	1329			0.534	3
3) +1.0 mm	507	35								
cluster I accepted (lateral C1)			42990	0.490	76	11020	1799	744	0.256	22
-"- II not accepted (medial C1)			2465	0.142	122	1450			0.588	3
4) +0.9 mm	428	11								
cluster I not accepted (lateral C1)			2779	0.164	111	991			0.357	2
-"- II not accepted (medial C1)			1088	0.105	76	580			0.533	1
5) -0.2 mm	185	21								
cluster VI accepted (part of nuc. solitarius)			18650	0.371	58	3504	549	2550	0.188	19

* estimated number of cells in each cluster.

ACKNOWLEDGEMENTS

This work has been supported by a grant (MH 25504) from the National Institute of Health. For excellent secreterial assistance we are grateful to Miss Elisabeth Sandqvist and to Mrs Anne Edgren.

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