the 2 homologues of pair 7 exhibited this distinctive feature, giving the appearance of a heteromorphic pair which may be confused with a sex chromosome pair. No clear heteromorphism in relation to sex chromosome pair has been noticed in either of the 2 sexes. In some cases the 8th pair was found to be slightly heteromorphic but it was not of universal occurrence. The arm ratio and relative percentage length are given in the table.

Uperodon globulosum belongs to the family Microhylidae which is considered as one of the most problematic families in amphibia[8,9]. So far as the present author is aware, only 1 more species belonging to genus *Uperodon (Cacopus)* has been studied cytologically. In his report on the chromosomes of *Cacopus systoma,* Natarajan[3] suggested 26 as 2n number in this species with NF value 50. This seems interesting when compared with *U. globulosum,* because 2n=26 with NF value 50 indicate the presence of a telocentric chromosome in the karyotype which seems to be very uncommon for the family Microhylidae[9]. In the karyotype of *U. globulosum,* no such telocentric pair has been noticed; but in certain cells the chromosome pair No. 10 showed the presence of 2 inconspicuous short arms giving the appearance of a telocentric element.

Spontaneous deviations from 2n number have been noticed in the present study (figure 2). Previously Sharma et al.[4], Yadav[10] and Chakrabarti[11] also reported the occurrence of subdiploid number in the somatic cells of different animals. Thus the fallacy of earlier speculations[5,12] that the hypodiploidy at mitotic level may be attributed to faulty techniques of squashing, etc., has been borne out by the present investigation.

Measurement of chromosomes of *U. globulosum* (SA, short arm; LA, long arm)

Arm ratio (index: SA/LA × 100)	Pair No.	Relative % length (as per 2n set)
69.23	1	65.28
68.00	2	62.30
59.09	3	53.40
52.17	4	51.92
61.90	5	50.44
78.57	6	37.09
90.90	7	31.15
75.00	8	31.15
90.90	9	31.15
41.66	10	25.22
87.50	11	22.25
75.00	12	20.77
71.42	13	17.80

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2 B.R. Seshachar, Cytologia *8*, 327 (1937).
3 R. Natarajan, Proc. 40th Indian Sci. Cong., part III, 180 (1953).
4 G.P. Sharma, R. Prashad and M.L. Gupta, Cellule *65*, 295 (1965).
5 G.K. Manna and S.P. Bhunya, Caryologia *19*, 403 (1966).
6 S. Chakrabarti, Genen Phaenen *16*, 1 (1973).
7 S. Chakrabarti and A. Chakrabarti, Experientia *33*, 175 (1977).
8 I. Griffiths, Biol. Rev. *38*, 241 (1963).
9 A. Morescalchi, in: Cytotaxonomy and vertebrate evolution. Ed. A.B. Chiarelli and E. Capanna. Academic Press, London/New York 1973.
10 J.S. Yadav, Experientia *30*, 1070 (1974).
11 S. Chakrabarti and A. Chakrabarti, Proc. 63rd Indian Sci. Cong., part III, 155 (1976).
12 K. Dutta, Experientia *22*, 815 (1966).

Absence of dopamine sensitive adenylate cyclase in the A10 region, the origin of mesolimbic dopamine neurones

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Summary. Dopamine (DA) failed to stimulate the adenylate cyclase of the mesolimbic A10 DA nerve cell body area, in contrast to its activating effect in the nigrostriatal A9 DA cell body area. The enzyme was stimulated by GMPPNP (a GTP analog) and NaF. This indicates the absence in the A10 cell area of DA receptors with functional coupling on adenylate cyclase, in contrast to the A9 cell area where such DA receptors are believed to be located on afferent axon terminals.

2 of the major dopamine (DA) neurone systems in rat brain are the nigrostriatal A9 and the mesolimbic A10 systems[2]. The A9 cell body-dendritic area, the substantia nigra, contains a DA-sensitive adenylate cyclase (EC 4.6.1.1.)[3,4]. This indicates the presence of DA receptors, since some DA receptors of nervous tissue are intimately associated with a DA-sensitive adenylate cyclase[5,6]. The adenylate cyclase-linked DA receptors of the substantia nigra appear to be located on striato- and/or pallidonigral axon terminals[7-9]. It has been reported that nigral A9 dendrites can release DA which interacts with these receptors[10,11]. The striato- and pallidostriatonigral axons, thought to be part of feedback systems which control nigral neurones, use substance P and γ-aminobutyric acid (GABA) respectively as transmitters[12,13].

We have studied the possible existence of a similar synaptic organization, involving DA receptors on feedback axons, in the cell body area of the mesolimbic A10 DA system, namely the ventral tegmental area of Tsai (VTA)[2,14]. The VTA was investigated for the presence of a DA-sensitive adenylate cyclase. Morphological and neurochemical studies have shown that at least 1 of the mesolimbic DA terminal regions, the nucleus accumbens, projects to the VTA among other regions[14,15]. In addition, the existence of a descending inhibitory system from the nucleus accumbens to the VTA is indicated by recent neurophysiological experiments[16]. The accumbal-VTA neurones do not contain substance P, but might contain GABA[15,16].

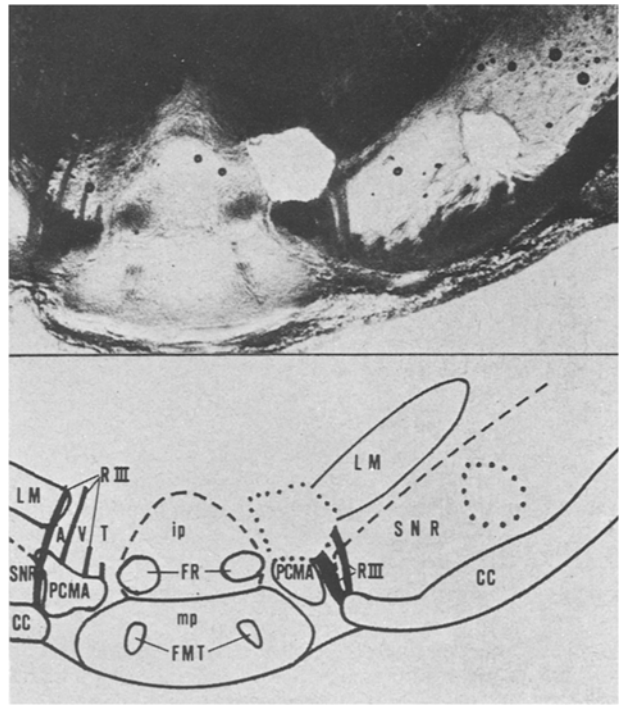
Materials and methods. Male outbred SPF rats (Füllinsdorf Albino stock) weighing 140–180 g were used. The A10 cell body area VTA was punched out with a stainless steel cannula (internal diameter 0.75 mm) from 250-µm-thick frontal brain sections according to a microdissection method[17], using the atlas of Palkovits and Jacobowitz[18].

For 5 samples, 3 sections from each of 30 rat brains were prepared and 3 (for sample 1) and 2 (for samples 2–5)

punches per section were removed from an approximate cranio-caudal area extending from A 2.30 to A 1.55 mm. For sample 6 one section only (A 2.20–A 1.95 mm) from each of 80 rat brains was prepared and 2 punches removed. 2 punches were always placed laterally (figure) and the 3 punch (if taken) mediodorsally to the interpeduncular or posterior mamillary nucleus. The medial punch contained very few A10 cells, much less than the lateral punches. Note that in sample 6 the 2 punches were confined to the most highly concentrated A10 cells (figure). In every section, the same number of punches was made in the substantia nigra zona reticulata as in the VTA with the same cannula (figure). 50 punches corresponded to approximately 11 mg protein determined colorimetrically[19]. The punches were blown out into 0.6 ml ice-cold Tris maleate 2 mM, pH 7.4 containing EGTA 2 mM and subsequently homogenized. The total homogenate was either preincubated for 10 min at 30 °C (for guanyl-5′-ylimidodiphosphate [GMPPNP], which is resistant to hydrolysis by nucleotide phosphohydrolases[20] and for NaF) and then incubated for 3 min at 30 °C with 0.5 mM ATP. DA was added together with ATP. The newly formed cyclic adenosine 3′,5′ monophosphate (cAMP) was determined according to Brown et al.[21].

Results and discussion. The table shows that DA did not significantly activate the adenylate cyclase in the VTA. However, using as activators of the adenylate cyclase GMPPNP and NaF, which are believed to act on the catalytic rather than the regulatory site of the enzyme[22], cAMP values of 234 and 201% respectively of control incubations were measured in the VTA. GMPPNP and NaF were preincubated, since these compounds activate the adenylate cyclase only slowly[23,24]. In marked contrast to the findings in the VTA, DA almost doubled the adenylate cyclase activity in the A9 cell body-dendritic area substantia nigra zona reticulata (table), as could be expected from earlier findings[4,8].

There are 2 possible explanations for the present findings. 1st that a difference exists in the synaptic organization between the mesolimbic A10 and the nigrostriatal A9 DA systems[8]. Nigral A9 dendrites are believed to release DA[10,11] which probably acts on feedback axons, where the adenylate cyclase-linked DA receptors of substantia nigra appear to be located[7–9]. The absence of the DA-sensitive adenylate cyclase in the VTA might indicate that such a DA mechanism is absent in the mesolimbic A10 system, although the existence of feedback axons terminating in the VTA, at least from the nucleus accumbens, is well documented[14,16]. Thereby we assume that the DA-sensitive adenylate cyclase exhibits similar properties in all brain regions, an assumption justified in the light of studies of DA-rich rat brain areas[3,5,7]. A 2nd possible interpretation of the findings is that the feedback axons in the VTA might bear DA receptors but that they are unrelated to adenylate cyclase, as they exist, e.g., in the anterior pituitary[6]. In fact, the existence of some type of DA receptors in the VTA is indicated by the finding that DA cells in the VTA are inhibited by microiontophoretic administration of DA and apomorphine[25] like those in the substantia nigra. However, in the nigra these effects are mediated by DA receptors on the DA cell bodies (autoreceptors) which do not regulate adenylate cyclase activity[6]. Thus, these and the present findings favour the existence of only DA autoreceptors in the VTA.



Unstained section corresponding approximately to plane A 2100[18] which contains the most highly concentrated A10 cell bodies. Typical punches are shown in the unilateral ventral tegmental area (AVT, A10 area; other side not punched to demonstrate topography) and in the substantia nigra zona reticulata (SNR, A9 area). If the A10 cell area was removed with 3 punches (for 1 of 6 samples), the 3rd punch was placed dorso-medially to the interpeduncular nucleus (i.p.).

CC: crus cerebri, FMT: fasciculus mamillothalamicus, FR: fasciculus retroflexus, LM: lemniscus medialis, mp: nucleus mamillaris posterior, PCMA: pedunculus corporis mamillaris, R III: radix n. oculomotorii.

Activation of adenylate cyclase by dopamine, NaF and GMPPNP in the ventral tegmental area (A10 cells) and substantia nigra zona reticulata (A9 cells and dendrites)

Agonist	Molar concentration	Preincubation	pmole cAMP formed/mg protein/3 min (30 °C)	
			VTA	Substantia nigra
None	–	–	300 ± 18**	454 ± 35
Dopamine	3×10^{-5}	–	313 ± 14 NS	820 ± 31*
Dopamine	10^{-4}	–	322 ± 22 NS	
Dopamine	3×10^{-4}	–	336 ± 41 NS	
None	–	+	221 ± 11**	
NaF	10^{-2}	+	443 ± 35*	
GMPPNP	10^{-5}	+	517 ± 66*	

The mean values with SEM of the 6 samples (for dissection see text) are given. * = $p < 0.001$ and NS = not significantly different from the control values (Kolmogorov-Smirnov test). The values of samples 1 (mixed approximate to highly concentrated A10 cell punches) and 6 (only punches with highly concentrated A10 cells) were within the 95% confidence limits of the values of samples 2–5 (mixed punches with moderately and highly concentrated A10 cells). ** Preincubation decreases adenylate cyclase activity.

Since the adenylate cyclase-linked DA receptors in substantia nigra are more concentrated in the dendritic (zona reticulata) than in the cell body area (zona compacta) of DA neurones[3,8], various series of VTA punches were investigated which included, to differing degrees, tissue around the area of highly concentrated DA cell bodies. Thereby, we attempted to include tissue possibly rich in DA dendrites and DA receptors but relatively deficient in DA cell bodies. Similar results were obtained with the various samples (single values not shown). A study of the distribution and morphology of the A10 dendrites, as well as of mesolimbic feedback axon terminals (other than those originating from the nucleus accumbens) would be desirable to substantiate the present conclusions.

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2 U. Ungerstedt, Acta physiol. scand., suppl. *367*, 1 (1971).
3 J.W. Kebabian and J.M. Saavedra, Science *193*, 683 (1976).
4 O.T. Phillipson and A.S. Horn, Nature *261*, 418 (1976).
5 Y.C. Clement-Cormier, J.W. Kebabian, G.L. Petzold and P. Greengard, Proc. natl Acad. Sci. USA *71*, 1113 (1974).
6 J.W. Kebabian, Life Sci. *23*, 479 (1978).
7 K. Gale, A. Guidotti and E. Costa, Science *195*, 503 (1977).
8 O.T. Phillipson, P.C. Emson, A.S. Horn and T. Jessell, Brain Res. *136*, 45 (1977).
9 P.F. Spano, M. Trabucchi and G. Di Chiara, Science *196*, 1343 (1977).
10 A. Björklund and O. Lindvall, Brain Res. *83*, 531 (1975).
11 L.B. Geffen, T.M. Jessell, A.C. Cuello and L.L. Iversen, Nature *260*, 258 (1976).
12 M.J. Brownstein, E.A. Mroz, M.L. Tappaz and S.E. Leeman, Brain Res. *135*, 315 (1977).
13 M.C. Olianas, G.M. De Montis, G. Mulas and A. Tagliamonte, Eur. J. Pharmac. *49*, 233 (1978).
14 W.J.H. Nauta, G.P. Smith, R.L.M. Faull and V.B. Domesick, Neuroscience *3*, 385 (1978).
15 J.L. Waddington and A.J. Cross, Life Sci. *22*, 1011 (1978).
16 P. Wolf, H.-R. Olpe, D. Avrith and H.L. Haas, Experientia *34*, 73 (1978).
17 M. Palkovits, Brain Res. *59*, 449 (1973).
18 M. Palkovits and D.M. Jacobowitz, J. comp. Neurol. *157*, 29 (1974).
19 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
20 Y. Salomon, M.C. Lin, C. Londos, M. Rendell and M. Rodbell, J. biol. Chem. *250*, 4239 (1975).
21 B.L. Brown, J.D.M. Albano, R.P. Ekins and A.M. Sgherzi, Biochem. J. *121*, 561 (1971).
22 N. Sahyoun, C.J. Schmitges, H. Le Vine and P. Cuatrecasas, Life Sci. *21*, 1857 (1977).
23 L.S. Bradham, J. cyclic Nucleot. Res. *3*, 119 (1977).
24 W.P. Burkard, Experientia *33*, 788 (1977).
25 G.K. Aghajanian and B.S. Bunney, in: Frontiers in catecholamine research, p.643. Ed. E. Usdin and S.H. Snyder. Pergamon, New York 1973.

The sparing effect of dose-fractionation in adult *Drosophila*

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Summary. The sparing effect of dose-fractionation was observed in adult male and female *Drosophila melanogaster;* 24-, 48-, 72-, and 96-h-old female flies, in general, show a higher recovery (increase in life-span) following dose-fractionation as compared to the males of the respective age. Recovery in 72-h-old females is maximal (31% increase in life-span) as against only 12% increase in the life span of the males.

Sparing effect of dose-fractionation is well documented in single cell systems like algae[2], yeast[3] and various types of cultured mammalian cells[4] as well as multicellular entities such as fish[5], larvae and imaginal stages of insects[6–9]. The presence of repair mechanism(s) in irradiated adult *Drosophila* has been deduced from experiments with fractionated doses[10]. In these experiments, a 'sparing effect' has been observed. Since adult male and female *Drosophila* show differential radiosensitivity towards gamma-rays[11–13], the present experiments were designed to study the kinetics of recovery in 24-, 48-, 72- and 96-h-old males and females separately.

Adult flies were exposed to gamma radiation in a 5500_{Ci} 60_{Co} gamma cell. The gamma radiation dose was 60 krad given either as a single dose, or as 2 equal fractions of 30 krad each separated by intervals of 6, 12, 18 and 24 h. Prior to irradiation, randomly selected samples of about 100 males and 100 females of the required age were sedated with ether and transferred to vials for irradiation. After the initial irradiation, the flies were immediately returned to the food vials. For a 2nd irradiation, the flies were transferred to small vials without using ether. Deaths were scored daily. The percentage mortality (initial number of adults/number of flies dead) (N/No.) was used to score the effect of treatment. Surviving flies were transferred to fresh food vials every eighth day.

The table shows the magnitude of recovery among females which is quite remarkable (31% increase in life span) as

Data showing percentage recovery (increase in life-span) adult *Drosophila melanogaster*

Time interval (between 2 fractionated doses)	Age of fly (h) Male				Female			
	24	48	72	96	24	48	72	96
6 h	Nil	Nil	4.1	Nil	17.2	20.0	29.6	23.0
12 h	9.5	4.6	Nil	Nil	12.4	20.0	26.4	20.5
18 h	Nil	Nil	4.1	Nil	28.1	25.0	31.2	30.7
24 h	1.1	Nil	12.3	Nil	18.9	30.0	20.0	29.0

The percentage recovery was calculated by comparing mortality following split dose (30 krad and 30 krad) with a dose-response curve produced by single exposure (60 krad) of adults of same age and treatment. Number of flies taken in each treatment = 300.