tissue is really free from ice crystals in the superficial zone, and if the sections are thin enough. In longitudinal sections of relaxed papillary muscle in the region of the cisternae we found concentrations of 67.2 mM Ca/kg dry wt ±2.8 (= 16.8 mM/1 SR) and in the sarcomere 6.8 mM Ca/kg dry wt ±0.8.

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Transplanted leg imaginal discs establish nerve connections with the appropriate neuromeres of the host's thoracic ganglion in the fly *Sarcophaga bullata*[1]

P. Sivasubramanian

Department of Biology, University of New Brunswick, Fredericton (New Brunswick, Canada E3B 5A3), 8 January 1979

Summary. Ectopic transplantations of prothoracic leg imaginal discs of mature 3rd instar larvae of *Sarcophaga bullata* on to young prepupae resulted in the development of supernumerary legs. The nerves that connected these legs with the host's CNS projected in the appropriate neuromere of the thoracico-abdominal ganglion.

The central question of developmental neurobiology is how the growing axons find their appropriate target cells on which to synapse. Though regenerating axons usually form proper connections with very high accuracy[2,3], the mechanism of formation of specific connections during normal development is far from clear. Studies with homeotic mutants of *Drosophila,* where an antenna is transformed into a leg structure, have revealed that the sensory neurons of these homeotic legs project to the antennal glomerulus of the brain and not to the leg projection areas of the thoracic ganglion[4,5]. In this report, I have taken advantage of a special transplantation technique[6] to study the development of nerve connections between the peripheral structures and the central nervous system in the fleshfly, *Sarcophaga bullata.*

Material and methods. Imaginal leg discs of mature 3rd instar larvae of the fleshfly *Sarcophaga bullata* were dissected out in sterile *Drosophila* Ringer's solution[7] and surface transplantations were made as described elsewhere[6,8]. Less than 3-h-old prepupae were used as hosts. When the host pupae metamorphosed, the transplanted leg discs also everted and differentiated as supernumerary legs on the host flies. The leg nerves were back-filled with 150 mM cobalt chloride solution[5,9], and the filled ganglia were processed and mounted in Canada balsam.

Results and discussion. Since the intent of this report is to locate the general projection area of the nerves of the transplanted legs, no attempt was made either to identify the individual neurons involved or to distinguish between the sensory and motor components in the projected area. Immersion of the cut ends of the in situ legs of the control flies into cobalt chloride solution resulted in the deposition of cobalt sulfide precipitate in the entire neuromere of the respective leg. There was no diffusion of cobalt chloride beyond the boundaries of the particular neuromere concerned. For the studies on the nerves of the supernumerary legs, transplantations of prothoracic leg discs were made on the posterior ventral surface of host prepupae. This resulted in the development of supernumerary legs on the ventral surface of the mid-abdomen of host flies. Of the 16 fully everted supernumerary legs that were filled with cobalt chloride, in 11 cases there was definite precipitation of cobalt sulfide in one of the prothoracic neuromeres; and in no instance were both the right and left prothoracic neuromeres simultaneously filled. There was no deposition of cobalt sulfide in the mesothoracic, metathoracic or abdominal neuromeres. The filling was always restricted to the prothoracic neuromeres, and that to only 1 of the 2 prothoracic neuromeres. In the remaining 5 thoracico-abdominal ganglia, the filling was so poor that definite localization of cobalt sulfide could not be noticed in any area of the ganglion.

The results of the present report demonstrate that the supernumerary legs differentiated from the ectopic transplantation of imaginal discs develop nerve connection with the host's CNS and that these nerves project in the appropriate areas of the thoracico-abdominal ganglion. The adult nerves of normal in situ legs, as well as those of homeotic mutants, have been suggested to develop on the principle of contact guidance by following the larval nerve route[10]. In the case of supernumerary legs, no such larval nerve connection exists between the transplanted imaginal disc and the host's CNS. How then the growing axons from these transplanted imaginal discs find the host's thoracico-abdominal ganglion which is far from the site of transplantation, is not clear at present. They may very well have followed some pre-existing abdominal nerve of the host or they might have reached the thoracico-abdominal ganglion by some kind of general attraction, independent of contact guidance as it happens with the pioneering embryonic nerves[11]. Upon reaching the vicinity of the host's CNS, the nerve from the prothoracic supernumerary leg always enters the ganglion close to its site of projection – the prothoracic neuromere.

Although the nerves from the homeotic wing or leg of the double mutant bithorax post bithorax of *Drosophila* do project in their proper area, namely, mesothoracic neuropile, these nerves enter the ganglion at the sites appropriate for haltere nerve or metathoracic leg nerve respectively[10,12]. In these studies, it was suggested that the growing axons follow certain specific 'trails' within the CNS in order to form proper projections. In the case of the growing nerves of the supernumerary legs reported here, no such suggestion need be proposed because these nerves enter the ganglion close to the site of their projection area.

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Acid excretion in young and adult Wistar Kyoto and spontaneously hypertensive rats[1]

M. Belledonne, J.M. Preuss and H.G. Preuss

Departments of Medicine and Pathology, Georgetown University School of Medicine, Washington (DC 20007, USA),

12 March 1979

Summary. Wistar Kyoto rats (WKy), the most widely accepted control for SH rats, show an inability to excrete acid appropriately when compared to another normotensive strain, SD. Coupled with the fact that KWy also develops 'sodium-sensitive' hypertension, this makes them a more complex control than realized. At very young ages (< 10-week-old), neither SH nor WKy show any deficiency in acid excretion.

When Okamoto and Aoki[2] first bred spontaneously hypertensive (SH) rats, they bred simultaneously a control normotensive group, now called Wistar Kyoto rats (WKy). Logically, most investigators have used WKy rats as control for SH rats.

In 1971, Louis et al.[3] pointed out 2 interesting findings from their study on SH rats: 1. young SH (study initiated when rats were prehypertensive) eventually developed hypertension despite consumption of a diet completely devoid of sodium, and 2. when fed excess sodium, SH markedly increased their hypertension[4]. Therefore, SH were 'sodium-sensitive' rats, i.e., hypertension develops or worsens rapidly with heavy sodium ingestion[5,6]. Because of this, they postulated that SH rats inherit 2 groups of autosomal alleles responsible for their hypertension - 1 set that allows sodium sensitivity and 1 set that allows elevated blood pressures in the absence of sodium. Recently, we found that WKy rats also develop hypertension after 1 week of ingesting 1% w/v sodium chloride in their drinking water (average blood pressure 150 mmHg in 10 rats)[7]. Therefore, we postulated that WKy rats inherit at least 1 group of the alleles described for SH, i.e., the 'sodium-sensitive' component.

In 1976, we reported that SH rats when compared to normotensive Wistar rats (American strain) and normotensive Sprague-Dawley SD rats excreted acid poorly in response to both acute and chronic acid loading[8]. We did not perform studies using WKy rats. Our main purpose in the present investigation was to expand these studies on acid excretion to include the WKy rats, the generally accepted control for SH. As a secondary gain, we followed acid excretion in WKy, SH, and SD rats when they were young or less than 10 weeks of age. At this age, SH have not yet become hypertensive.

Methods. Experiments were carried out in 2 stages - first on older adult rats and later on younger rats. The younger rats had an average age of 6-10 weeks, while the older rats were between 20 and 25 weeks of age. The average weights of the younger rats were: SD 182 g± 6 (SEM); WKy, 185 g± 18 (SEM); and SH, 181 g± 12 (SEM). In adult rats, the average weights were: SD, 425 g± 12.5 (SEM); WKy, 305 g± 7.6 (SEM); and SH, 332 g± 6.6 (SEM). Both strains of Wistar rats were obtained from Taconic Labs, Germantown, New York, and the SD, from Flow Labs, Dublin, Virginia. The rats ate rat chow and drank water ad libitum. The procedures for giving acid loads and measuring CO_2

Table 1. Acid excretion in adult WKy, SH and SD rats

Rat	Number	Serum CO_2 content (mEq/1)	Urine Volume (ml/4 h)	pH	Titratable acid (μEq/h/100 g b.wt)	Ammonium (μm/h/100 g b.wt)
Water load						
SD	(17)	28.2±0.6	7.4±0.6	7.1±0.4	-	6.0±0.7
WKy	(29)	28.3±0.8	4.3±0.3[a]	7.1±0.1	-	7.2±0.8
SH	(26)	28.5±0.5	4.9±0.3[a]	7.3±0.1	-	5.3±0.5
Acute acid challenge						
SD	(19)	19.3±0.7	10.3±1.0	5.9±0.04	9.5±0.9	31.3±1.3
WKy	(19)	20.2±1.3	4.9±0.3[a]	5.7±0.03	10.8±0.9	25.9±0.7[a]
SH	(21)	20.9±1.3	5.8±0.7[a]	5.7±0.04	12.6±1.1	24.7±0.7[a]
Chronic acid challenge						
SD	(19)	21.4±0.6	7.6±0.5	5.6±0.04	11.9±2.0	67.6±2.1
WKy	(14)	19.9±1.3	5.1±0.3[a]	5.7±0.03	7.1±0.9[b]	59.7±2.6[b]
SH	(16)	21.7±0.8	5.4±0.4[a]	5.7±0.03	7.3±0.6[b]	52.9±2.0[a]

SD = Sprague-Dawley, WKy = Wistar Kyoto, SH = spontaneous hypertensive, values are means ± SEM.

[a] $p<0.01$
[b] $p<0.02$ compared to SD under same conditions.