teers telephoned they were told the rationale and plan of the experiment and asked about their age, medical history, and any recent drug use. Women were also asked to give detailed information on their menstrual cycle. Interested volunteers were scheduled for a clinical interview to establish sexual orientation, past and current sexual history and attitudes, and general psychological state. Volunteers who gave their informed consent became subjects and were scheduled for a series of visits for blood sampling. Appropriate Institutional Review Board guidelines for human research subjects were followed and confidentiality was guaranteed. Subjects were paid for their participation in the 6-day sampling regime. Subjects in all groups came from a variety of occupations: health care professionals, educators, paralegal assistants, clerical and technical personnel, laborers, homemakers, sales people, and students. They appeared to be in good health and to be free of psychiatric and drug problems at the time of their participation. All subjects were Caucasians 21 to 37 years of age. To be included in the homosexual sample, men had to have had erotic fantasies exclusively or primarily involving male partners since puberty (a rating of 5 to 6 on the Kinsey scale of sexual orientation) [A. C. Kinsey, W. B. Pomeroy, C. E. Martin, *Sexual Behavior in the Human Male* (Saunders, Philadelphia, 1948)]. Similarly, genital experiences had to have exclusively or primarily involved a male since puberty. Individuals included in the heterosexual sample reported having had erotic fantasies about and genital sexual contact with persons of only the opposite sex since puberty (Kinsey rating of 0). Thus, there was no overlap in fantasies or behavior between the heterosexual and homosexual samples. Groups were matched for comparable age distributions (range, 21 to 37; median age, 26). A clinical interview was used to classify all volunteers, the records being reviewed under blind conditions by all three authors. Subjects were included for analysis only if consensus was obtained as to their Kinsey ratings. This was especially critical for the integrity of the homosexual sample. Homosexuality is a term that covers a wide range of childhood and adult experiences. To establish a group of fairly exclusively homosexual men, it was essential to distinguish volunteers having exclusively homosexual desires, interests, and experiences lifelong from men having such characteristics intermittently, periodically, or only recently. Of 150 volunteers, 90 met age, health, and substance use criteria. Of these, 70 eventually met criteria for inclusion in a group and agreed to participate as subjects. Several withdrew from the study because of scheduling conflicts, problems in giving blood samples, irregularities in menstrual cycling, illness, or undisclosed reasons. Of the 55 subjects participating throughout the study, data on several were excluded from statistical analysis because of abnormal baseline hormone concentrations, leaving a final sample size of 43 subjects.

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12. Blood was allowed to clot at room temperature for 30 minutes, then refrigerated (4°C) for 1 hour. Serum was stored frozen (−20°C) for later analysis. All samples from a given subject were assayed for a given hormone within a single run to minimize interassay variability. Radioimmunoassay for LH, in a preparation calibrated against appropriate reference preparations, was determined by a modified radioimmunoassay method [W. D. Odell *et al.*, *J. Clin. Med.* **70**, 873 (1967)]. Intra- and interassay variance for this method were 6.3 and 9.5 percent, respectively. Estrone was determined with a direct coated-charcoal radioimmunoassay by using a modified method of N. Jiang, R. J. Ryan, and A. Albert [*Clin. Chem.* **19**, 470 (1973)] with an antibody specific for estrone (antibody to estrone-6-thyroglobulin; Miles-Yeda). In addition to binding estrone, this antibody binds constituent steroids of Premarin, notably equilin (4.6 percent) and equilenin (14.5 percent), while not cross-reacting with any androgens or other estrogens. Plasma concentrations of testosterone were determined by coated charcoal radioimmunoassay with an antibody highly specific for testosterone (anti-T-3-oxime-BSA; a gift of G. Bolleli), which cross-reacts with androstenedione (<0.05 percent; all other, <0.01 percent). This method, a modification of that reported by F. Kohen, S. Bauminger, and H. R. Lindner [in *Steroid Immunoassay*, E. H. D. Cameron, S. G. Hillier, K. Griffiths, Eds. (Alpha Omega, Cardiff, Wales, 1975), pp. 11–32] requires no chromatography, a simple extraction with petroleum ether:ethyl acetate (5:2 by volume), and is routinely used in our laboratory. Intra- and interassay variances were 7.4 and 9.5 percent, respectively.
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21 November 1983; accepted 25 July 1984

Plasticity of Substance P in Mature and Aged Sympathetic Neurons in Culture

Abstract. *The effect of age on the plasticity of the putative peptide neurotransmitter substance P (SP) was examined in the rat superior cervical sympathetic ganglion. Explantation of ganglia from 6-month-old rats to serum-supplemented culture resulted in a tenfold increase in SP concentration, reproducing results previously obtained for ganglia from neonatal rats. Veratridine prevented the increase in SP concentration in adult ganglia, and tetrodotoxin blocked the veratridine effect, suggesting that membrane depolarization and sodium influx prevented the rise in the SP content of adult ganglia as well as of neonatal ganglia. However, the time courses of the increase in the amount of the peptide differed in neonatal and mature ganglia, suggesting that some aspects of regulation may differ in the two. The effects of aging on neural plasticity were further analyzed by explanting ganglia from 2-year-old rats. No significant increase in SP concentration was observed in these ganglia. Remarkable plasticity thus seems to persist in mature neurons but may be deficient in aged sympathetic neurons.*

Evidence indicates that developing neurons are remarkably plastic, altering transmitter metabolism and even phenotypic expression in response to extracellular stimuli (*1–3*). Sympathetic neurons, long regarded as noradrenergic, may express cholinergic characters or the putative peptide transmitter substance P (SP) in vivo and in vitro during the neonatal period (*2–5*). However, whether transmitter plasticity is restricted to the developing neuron or whether it persists during maturity is unclear (*6*). Transmitter plasticity may play a role in the function of the adult nervous system.

To investigate this issue, we examined sympathetic neurons in the adult rat superior cervical ganglion (SCG). Details of the preparation and characteristics of antiserum to SP as well as the radioimmunoassay procedure have been described (*7*); the authenticity of immunoreactive SP was documented by high-performance liquid chromatography (*3*). Earlier studies have indicated that denervation (decentralization) of the neonatal rat SCG in vivo or its explantation to culture (with consequent denervation) markedly increased the amount of SP in principal sympathetic neurons (*3*). To determine whether explantation also increased SP in the adult rat SCG, we grew ganglia from 6-month-old rats under a variety of conditions for 1 week (Fig. 1). All groups of adult ganglia exhibited at least an eightfold increase in SP concentration, whether they were cultured in fully defined or serum-supplemented medium on buffer- or ammonia-reconstituted collagen (Fig. 1).

Depolarization of neonatal ganglia in culture is known to prevent the increase in SP concentration upon explantation (*3*). To determine whether depolarization similarly affects adult ganglia, we exposed explants to the depolarizing agent veratridine (*8*). Veratridine exposure completely blocked the increase of SP concentration in adult ganglia, reproducing the effect observed in neonatal ganglia (Fig. 2). Moreover, tetrodotoxin, which prevents the transmembrane sodium influx elicited by veratridine (*9, 10*), completely blocked the effect of veratridine (Fig. 2). Thus depolarization with attendant sodium influx prevented the increase in SP concentration in mature ganglia as it did in developing ganglia.

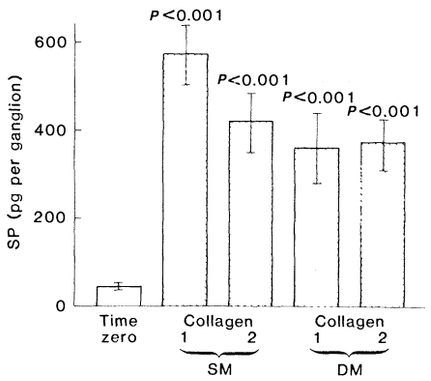
To characterize more fully the regulation of SP concentration in adult ganglia, we compared the time courses of the increases of the SP content of adult and neonatal ganglia. Although both adult and neonatal ganglia exhibited approximately tenfold increases in SP concentration after 7 days in culture, the temporal profiles of these increases differed markedly. In the neonatal ganglia the rise in SP concentration began virtually immediately, attaining a plateau after only 48 hours in culture (Fig. 3). In contrast, the amount of SP in adult ganglia increased relatively gradually, reaching only 18 percent of its final con-

centration after 3 days. Most of the increase in SP concentration occurred between 3 and 7 days in culture. Therefore, although the amount of SP increased in adult as well as in neonatal ganglia, some aspects of regulation may differ with age.

To analyze further the effect of aging on neural plasticity, we examined ganglia from 2-year-old rats. Basal peptide content of these ganglia was indistinguishable from that in adults, 30 pg per ganglion. However, in contrast to neonatal and mature ganglia, those from 2-year-old rats did not exhibit a statistically significant increase in SP concentration (Fig. 4). Mature ganglia attained a significantly higher (fourfold) increase in SP content than did aged ganglia after 1 week in culture (Fig. 4). The lack of an increase in SP in aged ganglia does not appear to have reflected a general deficit, because neuritic elaboration and immunocytochemistry of tyrosine hydroxylase (data not shown) were normal. Thus aged ganglia exhibited an apparently specific defect in the plasticity of the SP response.

Our observations indicate that striking transmitter plasticity, exemplified by an increase of an order of magnitude in SP concentration, occurs in adult as well as developing sympathetic ganglia. Since the peptide is contained within sympathetic neurons in the ganglia (*3, 11*), mature neurons must be profoundly mutable. Therefore, neuronal transmitter plasticity is not restricted to the developmental period but may be expressed during adulthood as well. Moreover, depolarization and transmembrane influx of sodium decreased the SP content of both adult and neonatal ganglia. A number of common molecular regulatory mechanisms therefore govern transmitter metabolism during development and maturity. More generally, mature neurons apparently remain intrinsically plastic. Nevertheless, the different time courses of the increases in SP concentration in adult and neonatal ganglia suggest that there are underlying differences between the two.

Although mature neurons remain markedly plastic, advanced age appears to interfere with this potential. We have not defined the basis of this difference between aged and mature (or developing) neurons. However, these observations raise the intriguing possibility that some deficiencies in the senile nervous system may be attributable to attenuation or loss of plastic responses.



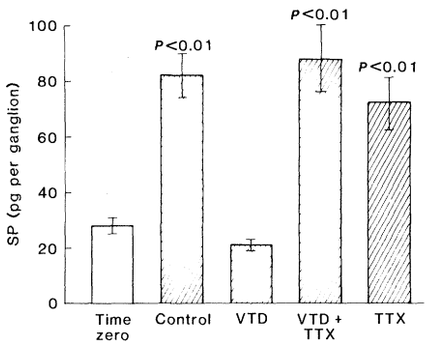
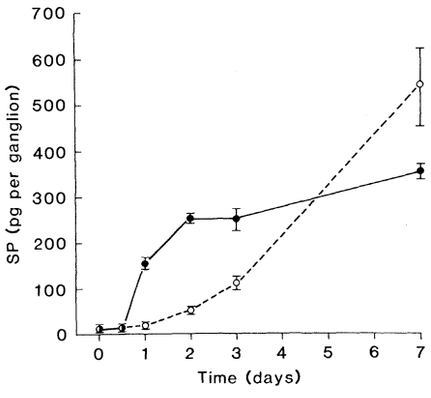


Fig. 1 (left). Accumulation of SP in adult rat ganglia. Superior cervical ganglia from rats older than 6 months were divided into 8 to 12 pieces and grown as explants on two substrates and in two media. After 1 week they were examined for their content of SP, which is expressed as the mean ± standard error of the mean for eight ganglia. All ganglia were grown at 37°C in an atmosphere of 95 percent air and 5 percent CO_2 at nearly 100 percent relative humidity. Collagen 1: single layer, air-dried, buffer-reconstituted; collagen 2: double layer, air-dried, ammonia-reconstituted; SM: Ham's nutrient mixture F-12 supplemented with human placental serum (10 percent), chick embryo extract (1 percent), glutamine (2 m*M*), glucose (0.6 percent), penicillin (50 unit/ml), and streptomycin (50 μg/ml); DM: Ham's nutrient mixture F-12 supplemented with transferrin (100 μg/ml), insulin (5 μg/ml), putrescine (100 μ*M*), sodium selenite (30 n*M*), progesterone (20 n*M*), penicillin (50 unit/ml), and streptomycin (50 μg/ml). Statistical analysis (difference from time zero) was by one-way analysis of variance (ANOVA) and Newman-Keuls test. Fig. 2 (right). Effect of membrane depolarization on SP content of adult rat ganglia. Ganglia were cultured in serum-supplemented medium in the presence of veratridine (VTD) (5×10^{-5} *M*) or tetrodotoxin (TTX) (10^{-7} *M*) or both. After 48 hours the ganglia were examined for their content of SP, which is expressed as the mean ± standard error of the mean for eight ganglia. Statistical analysis (difference from time zero and veratridine) was by one-way ANOVA and Newman-Keuls test.



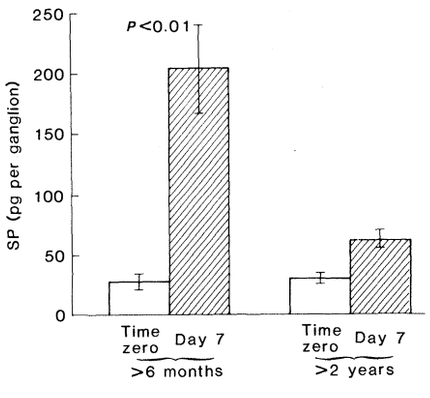


Fig. 3 (left). Time course of SP accumulation in adult (○) and neonatal (●) rat ganglia. Ganglia were grown in serum-supplemented medium for varying periods of time and examined for their content of SP, which is expressed as the mean ± standard error of the mean for 16 ganglia per point. Fig. 4 (right). Accumulation of SP in adult (older than 6 months) and aged (older than 2 years) rat ganglia. Ganglia were grown in serum-supplemented medium. After 1 week their content of SP was determined and expressed as the mean ± standard error of the mean for 16 ganglia. Statistical analysis (difference from all other groups) was by one-way ANOVA and Newman-Keuls test.

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12. This work was supported by grants NS 10259 and HD 12108 from the National Institutes of Health and by a grant from the March of Dimes Birth Defects Foundation. We thank D. Straka, E. Grossman, and B. Mayer for technical assistance.

10 May 1984; accepted 2 July 1984