J1/tenascin in Substrate-bound and Soluble Form Displays Contrary Effects on Neurite Outgrowth

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Abstract. The influence of J1/tenascin adsorbed to polyornithine-conditioned plastic (substrate-bound J1/tenascin) and J1/tenascin present in the culture medium (soluble J1/tenascin) on neurite outgrowth was studied with cultured single cells from hippocampus and mesencephalon of embryonic rats. Neurons at low density grew well on J1/tenascin substrates and extended neurites that were ∼40% longer than on the polyornithine control substrate after 24 h in vitro. The neurite outgrowth promoting effect of substrate bound J1/tenascin was largely abolished in the presence of mAb J1/tn2, but not by mAb J1/tn1. In contrast to the neurite growth–promoting effects of substrate bound J1/tenascin, neurite outgrowth on polyornithine, laminin, fibronectin, or J1/tenascin as substrates was inhibited by addition of soluble J1/tenascin to the cultures. Neither of the two mAbs neutralized the neurite outgrowth–inhibitory properties of soluble J1/tenascin. In contrast to their opposite effects on neurite out-growth, both substrate-bound and soluble J1/tenascin reduced spreading of the neuronal cell bodies, suggesting that the neurite outgrowth–promoting and anti-spreading effects are mediated by two different sites on the molecule. This was further supported by the inability of the mAb J1/tn2 to neutralize the antispread-ing effect. The J1/tn2 epitope localizes to a fibronectin type III homology domain that is presumably distinct from the putative Tn68 cell-binding domain of chicken tenascin for fibroblasts, as shown by electronmicro-scopic localization of antibody binding sites. We infer from these experiments that J1/tenascin contains a neurite outgrowth promoting domain that is distinguishable from the cell-binding site and presumably not involved in the inhibition of neurite outgrowth or cell spreading. Our observations support the notion that J1/tenascin is a multifunctional extracellular matrix molecule.

THE J1-glycoproteins of 160- and 180-kD (J1-160/180) and 200- and 220-kD (J1-200/220) apparent molecular weights were originally described as constituents of the L2/HNK-1 family of neural cell adhesion molecules (Kruse et al., 1984, 1985). Polyclonal J1 antibodies localized the J1-glycoproteins to the surface of glial cells and inhibited the binding of small cerebellar neurons to astrocyte monolayers in vitro (Kruse et al., 1985). Subsequent studies with specific mono- and polyclonal antibodies have shown that J1-160/180 and J1-200/220 have to be considered as two independent molecular pairs which share a small, but detectable degree of homology (Faissner et al., 1988; Pesheva et al., 1989; Faissner and Kruse, 1990). J1-160/180 is an extracellular matrix molecule associated with oligodendrocytes in postnatal mouse central nervous system (Pesheva et al., 1989) and is involved in neuron–oligodendrocyte and astrocyte–oligodendrocyte interactions (Morganti et al., 1990). As substrate in mixture with laminin purified J1-160/180 in-terferes with attachment and neurite outgrowth of cerebellar granule cells (Pesheva et al., 1989).

J1-200/220 is the mouse equivalent of tenascin, previously designated myotendinous antigen (Chiquet and Fambrough, 1984a,b; Chiquet-Ehrismann et al., 1986) as revealed immunochemically and by primary sequence analysis (Faissner et al., 1988; Weller et al., 1991). It belongs to a group of extracellular matrix (ECM)[1] molecules which includes the hexabrachion (Erickson and Inglesias, 1984), glioma mesen-chymal extracellular matrix antigen (Bourdon et al., 1985), cytotactin (Grumet et al., 1985), and polypeptide 150/225 (Gulcher et al., 1986). Tenascin and related molecules form multimers linked by NH_2-terminal disulfide bridges thereby creating the so-called T-junction (Erickson and Inglesias, 1984). Molecular genetic investigations have shown that

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1. *Abbreviations used in this paper:* ECM, extracellular matrix; N-CAM, neural cell adhesion molecule.

tenascin from chicken mesenchyme consists of at least three isoforms which contain variable numbers of fibronectin type III repeats, a motive originally described in fibronectin (Petersen et al., 1983), a cystein-rich aminoterminal domain followed by 13.5 EGF-like repeats, a motive also present in laminin (Sasaki and Yamada, 1987; Sasaki et al., 1987), and a carboxyterminal fibrinogen homology domain (Jones et al., 1989; Spring et al., 1989). The human hexabrachion displays ~80% homology to chicken tenascin (Gulcher et al., 1989).

J1/tenascin exhibits a site- and time-restricted expression in various developing organs. It displays different functional properties when isolated from developing mesenchymal and neuroectodermal sources (Faissner et al., 1990; Probstmeier et al., 1990) and is barely detectable in the adult tissues investigated (for review see Erickson and Bourdon, 1989). J1/tenascin is synthesized by cultured astrocytes and Schwann cells (Kruse et al., 1985; Faissner et al., 1988; Seilheimer and Schachner, 1987). In the peripheral nervous system, cytotactin (Daniloff et al., 1989), and J1/tenascin (Martini et al., 1990) are reexpressed in the regenerating adult sciatic nerve. In the cerebral cortex, J1/tenascin immunoreactivity delineates the somatosensory barrel fields which have been proposed to be demarcated by transitory glial boundaries during the period of sensory afferent ingrowth (Crossin et al., 1989; Steindler et al., 1989a,b).

Since J1/tenascin is expressed in the nervous system at critical developmental time periods and may occur both in membrane-associated and soluble forms, we attempted to investigate the influence of both forms of J1/tenascin on neuronal differentiation. To this aim, central nervous system neurons were cultured at low density on J1/tenascin adsorbed to polyornithine-coated plastic or on different substrates in the presence of soluble J1/tenascin, and neurite outgrowth and cell body spreading were analyzed morphometrically. We show here that J1/tenascin promotes neurite outgrowth and elongation when used as substrate, but that it inhibits neurite extension on all substrates tested when added to the culture medium in soluble form. We localize a neurite outgrowth–promoting region on the molecule and propose that it is different from the cell-binding site for fibroblasts (Spring et al., 1989) and not involved in inhibition of neurite outgrowth and cell body spreading.

Materials and Methods

Animals

For the preparations of single cells from embryonic brains, CD1 rats were used. The day a vaginal plus was found was designated embryonic day 0 (E0).

Analytical Procedures

Protein concentrations were measured according to Bradford (Bradford, 1976). SDS-PAGE was performed on 4–10% gradient slab gels (Laemmli, 1970). The gels were stained with reducing silver ions (Merril et al., 1982).

Antibodies

The rat monoclonal IgG antibodies J1/tn1 and J1/tn2 against J1/tenascin have been described (Faissner and Kruse, 1990; Steindler et al., 1989a; Stern et al., 1989). The hybridoma clones were grown in RPMI 1640 containing Nutridoma (Boehringer Mannheim Biochemicals, Indianapolis, IN) and used as concentrated supernatants. SDS-PAGE revealed a purity of >95% of the antibodies. Polyclonal antibodies to neural cell adhesion molecule

(N-CAM) (Trotter et al., 1989) were a kind gift of Dr. J. Trotter and peroxidase-coupled secondary antibodies to rabbit IgG were purchased from Cappel Laboratories (Cochranville, PA).

ECM Molecules

Human serum fibronectin and the GRGDS peptide were purchased from Boehringer Mannheim Biochemicals. Laminin isolated from Engelbreth-Holm-Swarm mouse sarcoma cells (Timpl et al., 1979) was a kind gift of Dr. K. Kühn (Max-Planck-Institut für Biochemie, Martinsried, Germany). J1/tenascin was immunoaffinity purified on J1/tn1 and J1/tn2 columns from detergent-free extracts of brains from zero- to 15-d-old mice (Hoffman et al., 1988; Faissner and Kruse, 1990). Human tenascin was prepared from supernatants of the U-251MG glioma cell line (Vaughan et al., 1987).

Preparation of Substrates

Cells were grown in 8-well tissue culture plastic chamber slides (Nunc, Roskilde, Denmark; 0.8 cm^2/well). For preparation of coated substrates, culture dishes were incubated for 1–2 h at 37°C with 1.5 μg/ml poly-DL-ornithine (Sigma Chemical Co., St. Louis, MO) in 0.1 M borate buffer, pH 8.2. The wells were washed twice with distilled water, air dried, incubated overnight at 37°C with PBS, pH 7.4 (PBS; 100 μl/well) or PBS containing 50 μg/ml J1/tenascin, laminin, or fibronectin, sterilized by passage through filters with low protein binding capacity (Millex GV4; Millipore/Continental Water Systems, Bedford, MA), and finally washed twice again with PBS. The efficiency of J1/tenascin adsorption to the polyornithine-coated culture dishes was determined by addition of ^{125}I-labeled J1/tenascin (Faissner et al., 1990) as tracer to the coating solution. Under the conditions of this study, 22% (1.1 μg) of the added J1/tenascin adsorbed to the bottom of the well. Of the adsorbed J1/tenascin 5% (0.1 μg) was found to be released into the culture medium during the following 24-h culture period.

Cell Culture

Hippocampal cell cultures were established from E18-E19 rat brains (Banker and Cowan, 1977) with some modifications. Hippocampi were obtained by microdissection, placed in Ca- and Mg-free (CMF) HBSS, freed from meninges and incubated in CMF-HBSS containing 0.25% wt/vol trypsin (Gibco Laboratories, Grand Island, NY; Bethesda Research Laboratories, Gaithersburg, MD) for 10 min at 37°C. After three washes in CMF-HBSS, hippocampi were dissociated by trituration in CMF-HBSS containing 1 mM Mg$_2$SO$_4$ and 0.025% wt/vol DNase I (Boehringer Mannheim Biochemicals) using fire-polished Pasteur pipettes. The resulting cell suspension was centrifuged at 80 g for 10 min at room temperature and the pellet resuspended in chemically defined medium (Rousselet et al., 1988; but without putrescine) consisting of DME/F12 (1:1; Gibco Laboratories, Bethesda Research Laboratories) 2.4 mg/ml NaHCO$_3$, 33 mM D-glucose, 2 mM L-glutamine, 25 μg/ml insulin, 100 μg/ml transferrin, 2 × 10^{-8} M progesterone, 3 × 10^{-8} M selenium, 0.1% (wt/vol) ovalbumin, 5 mM Hepes, 5 IU/ml penicillin, and 5 μg/ml streptomycin. Monolayer cultures from single cell suspensions from mesencephalon and striatum were prepared from E14-E15 and E15-E16 rats, respectively, and plated in defined medium (Chamak and Prochiantz, 1989). 90% of the mesencephalic and 98% of the hippocampal cells were viable as judged by trypan blue exclusion. All cells were plated at a density of 10,000 cells/cm^2 in 250 μl medium per well and maintained in this medium for 24 h. The plating efficiency of hippocampal neurons 1.5 h after plating was ~70% on polyornithine alone and on polyornithine with substrate-bound J1/tenascin. Under both conditions 24 h after plating the number of cells attached to the culture substrate was only slightly decreased to about 65%. Viability of cells as determined by trypan blue exclusion after 24 h of culture was 60% on polyornithine alone and 80% on polyornithine with substrate-bound J1/tenascin, laminin, or fibronectin. Cultures of both hippocampal and mesencephalic cells were estimated to be 98% pure neurons by morphological (Banker and Cowan, 1977; for hippocampal cultures) or immunological (Chamak and Prochiantz, 1989; for mesencephalic cultures) criteria. Cells were also cultured in the presence of J1/tenascin, antibodies to J1/tenascin, or GRGDS peptide (Boehringer Mannheim). The proteins were dialyzed against DME/F12 and sterilized by filtration (Millex GV4; Millipore/Continental Water Systems) before addition to the cultures 1 h after cell plating at final concentrations of 15 and 60 μg/ml, and 2 mg/ml, respectively, unless otherwise indicated. For some experiments J1/tenascin was heat inactivated by boiling for 10 min. To test the influence of mAbs on the effects of substrate-bound J1/tenascin, the wells were blocked for 2 h at 37°C with 0.5 mg/ml heat-inactivated (5 min at

80°C) BSA in PBS, washed twice with PBS, incubated for 2 h at 37°C with 60 μg/ml of antibodies in PBS, and finally washed twice with PBS before cell plating. An equal concentration of antibodies was added to the culture medium 1 h after plating. When antibodies were tested for their influence on soluble J1/tenascin, they were incubated together with soluble J1/tenascin for 2 h at 37°C and added to the cultures 1 h after cell plating at final concentrations as indicated for the individual components. Addition of soluble J1/tenascin did not detach the cells from their substrate. Neither soluble J1/tenascin nor antibodies affected the survival rate of hippocampal neurons. To determine how much of the soluble J1/tenascin in the culture medium remained in solution or was adsorbed to the substrate, ^{125}I-labeled J1/tenascin was added as tracer to the culture medium. 75% (2.8 μg) of J1/tenascin remained in solution, 16% (0.6 μg) adsorbed to the bottom and 9% (0.3 μg) to the wall of each well after a 24-h culture period. To evaluate whether a selection for particular neuronal cell types occurred on the different substrates and under the different culture conditions, hippocampal cells were first maintained for 24 h in defined medium, detached from the substrate by incubation with 0.05% wt/vol trypsin and 5 mM EDTA in CMF-HBSS for 5 min at 37°C, collected by centrifugation, and maintained for another 24 h under different culture conditions.

Cell–Substrate Adhesion Assay

To test the influence of J1/tenascin on cell–substrate adhesion, 4,000 hippocampal neurons were plated in 20 μl of chemically defined medium per well in Terasaki dishes (Nunc) and cultured as described above on polyornithine (control), on substrate-bound J1/tenascin or in the presence of J1/tenascin added to the culture medium immediately after plating. After 2 or 24-h incubation dishes were flicked upside down on a solid support covered with paper towel and washed once with 25 μl chemically defined medium per well. Subsequently, residual cells were fixed and stained with toluidine blue as described below. Results were quantified by counting the number of cells attached to the flat bottom of individual Terasaki wells. When no mechanical shearing forces were applied, the plating efficiency after 2 h was 679 ± 61.7 cells (n = 6) under all conditions.

Histo- and Immunochemical Staining of Cultured Cells

For histochemical staining, cultures were fixed in PBS containing 2.5% (vol/vol) glutaraldehyde for 1 h at room temperature. After two washes with PBS, cells were stained for 15 min with 0.5% (wt/vol) toluidine blue (Sigma Chemical Co.) in 2.5% (wt/vol) Na_2CO_3, washed twice with distilled water, and air dried. For immunochemical staining, cultures were fixed with 4% paraformaldehyde in PBS for 15 min at room temperature, washed twice with PBS, and blocked for 10 min with 10% (vol/vol) horse serum in PBS. Cultures were then incubated for 30 min at 37°C with polyclonal N-CAM antibodies (1:200) in PBS containing 10% (vol/vol) horse serum, washed twice with PBS, and incubated for 30 min at 37°C with horseradish peroxidase–coupled secondary antibodies to rabbit IgG (1:1000) in PBS containing 10% (vol/vol) horse serum. Subsequently, the cells were washed twice with PBS, incubated with 0.5 mg/ml diaminobenzidine, 0.015% (vol/vol) H_2O_2 in 10 mM Tris-HCl, pH 7.4, at room temperature, washed twice with distilled water, and then air dried.

Morphometry and Statistical Analysis

For quantitative morphometry only singly growing cells were measured and only neurites exceeding one cell diameter in length were taken into account. Process bearing cells were determined as the fraction of at least 100 cells per well chosen at random and given in percent. Neurons extending processes were analyzed by evaluating the number of neurites and total length of neurites per cell and the surface area of the cell body. For determination of the time course of neuronal spreading, process bearing neurons as well as neurons without processes were analyzed. Surfaces and process lengths were measured using an Ai Tectron image analysis system (VIDS III software, Bestobell Mobrey GmbH, 4000 Düsseldorf, NRW, Germany). Samples of 50 randomly selected neurons were investigated per well. Since the single values obtained were not normally distributed, values of 50 neurons were summed up to apply a parametric comparison test among means for the determination of significance levels. The summed values exhibited normality, according to the G-test for goodness of fit and homoscedasticity according to the Bartlett's test of homogeneity, thus allowing statistical evaluation with a parametric planned comparison test (least significant differ-

ence- or LSD-test). Neurite lengths of individual neurons were additionally analyzed with the chi-square test for goodness of fit by grouping measurements obtained from single neurons into classes of equal length. In some cases, classes were lumped to obtain a theoretical frequency of at least three. The Williams' correction was applied to reduce the type I error. All statistical tests were taken from Sokal and Rohlf (1981). Plus/minus values appearing in the text or figure legends represent the respective SD. Graphical representations of results were performed on a MAC II PC using the programs Statview II TM (Abascus Concepts Inc., Berkeley, CA), Cricket Graph TM (Cricket Software, Malvern, PA), and Canvas TM (Deneba Software Inc., Miami, FL) at the ZMBH, University of Heidelberg.

Rotary Shadowing and Localization of Epitopes by EM

EM was performed as described (Vaughan et al., 1987). All samples were dialyzed against several changes of 0.2 M ammonium bicarbonate before use and were mixed to give final concentrations of 10–50 μg/ml antibodies and 50 μg/ml J1/tenascin. After incubation for 1–4 h at room temperature, each sample was diluted 2:1 (vol/vol) with glycerol, vortexed, and sprayed onto the surface of a freshly cleaved mica chip. Chips were affixed to the rotating stage of a freeze-fracture unit (model BAF300; Balzers AG, Lichtenstein), dried under a vacuum of better than 10^{-7} T for 4–6 h and shadowed with platinum/carbon at 7[a] followed by a backing film of carbon at 90[a]. Electron micrographs were obtained with an Hitachi S-600 electron microscope and calibration was by means of negatively stained catalase crystals. The curvilinear lengths of hexabrachion arms from the T-junctions to the center of the distal knob and the antibody-binding sites were measured with a graphics tablet interfaced with the Kontron IBAS graphics system (Kontron Elektronik GmbH, Zürich, Switzerland).
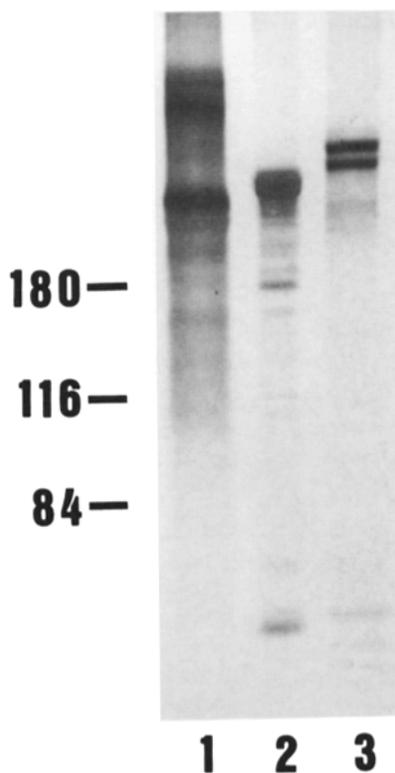

Figure 1. SDS-PAGE of purified laminin, fibronectin, and J1/tenascin. Laminin (lane *1*), fibronectin (lane *2*), and J1/tenascin (lane *3*), each at 2 μg, were resolved on a 4–10% polyacrylamide gradient slab gel under reducing conditions. Protein bands were visualized by the reducing silver method. Molecular weight markers are indicated (180 kD, $α_2$-macroglobulin; 116 kD, β-galactosidase, 84 kD, fructose 6-phosphate kinase).
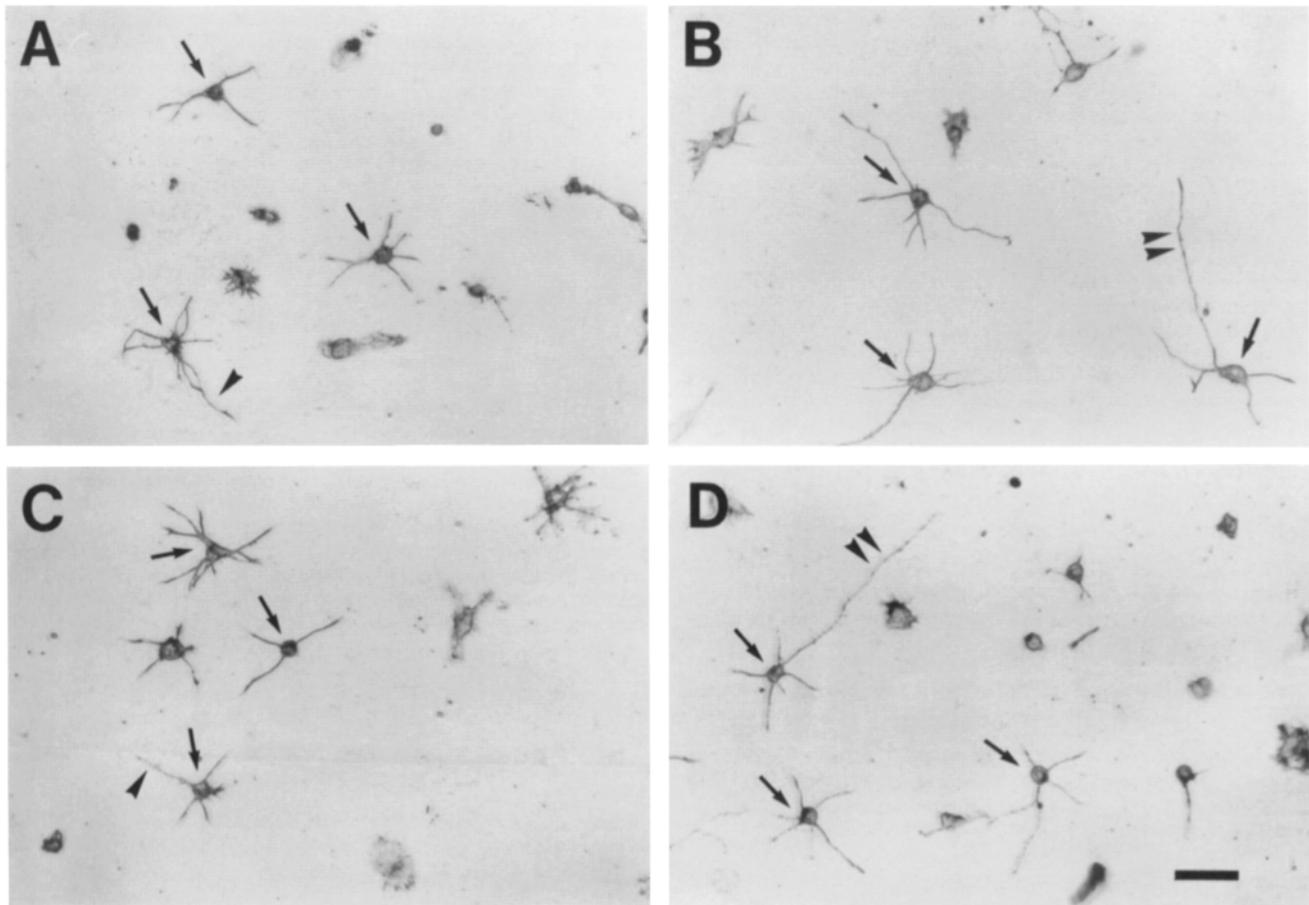
Figure 2. Morphology of hippocampal neurons cultured on substrate-bound J1/tenascin. Bright-field micrograph of hippocampal neurons cultured for 24 h on polyornithine (*A*, control), or J1/tenascin adsorbed to polyornithine-coated plastic in the absence (*B*) or presence of mAbs J1/tn2 (*C*) or J1/tn1 (*D*). Cells were stained with N-CAM antibodies by indirect immunoperoxidase. Typical process-bearing neurons are marked by arrows. Note the elaboration of single long axonlike processes in *B* and *D* (*double arrowheads*) as compared to the short ones in *A* and *C* (*arrowheads*). Bar, 20 μm.

Results

Substrate-bound J1/tenascin Promotes Neurite Extension

The influence of J1/tenascin on neurite extension was studied by maintaining hippocampal neurons at low density on defined substrates under serum-free conditions. In most experiments, cells were processed for morphometric analysis 24 h after plating, after which time 50% of the hippocampal neurons had developed processes on polyornithine-coated plastic, the control substrate. Of these neurons 80% exhibited 1–4 and 20% 5–10 neurites. To study the effects of J1/tenascin on neurite outgrowth on polyornithine, J1/tenascin was isolated from postnatal mouse brain. These preparations consisted of two major glycoproteins of 225 and 240 kD migrating ahead of fibronectin in 4–10% gradient gels, as described (Faissner and Kruse, 1990; Fig. 1, lanes 2 and 3). Hippocampal neurons grown on J1/tenascin adsorbed to polyornithine-coated plastic (substrate-bound J1/tenascin) exhibited a more elaborate morphology than on polyornithine alone (Fig. 2, *A* and *B*). The fraction of process-bearing cells and their total neurite length were increased by ~40% on substrate-bound J1/tenascin when compared to the control

(Fig. 3). These effects of the substrate-bound J1/tenascin were largely abolished by boiling the molecule before coating (not shown). Neurons cultured in the presence of the pentapeptide GRGDS, which blocks the interaction between integrins and some ECM molecules (Ruoslahti and Pierschbacher, 1987), were not modified in their outgrowth behavior on control or J1/tenascin substrates (not shown). Two mAbs against J1/tenascin, J1/tn1, and J1/tn2, were tested for interference with the effects of substrate-bound J1/tenascin. J1/tn2 strongly decreased outgrowth and elongation of neurites on substrate-bound J1/tenascin (Figs. 2 *C* and 3), whereas J1/tn1 did not significantly affect neurite extension (Figs. 2 *D* and 3). When neurite lengths on the control substrate or on substrate-bound J1/tenascin were compared in cumulative frequency distribution plots, they were continuously shifted to higher values on substrate bound J1/tenascin (Fig. 4), suggesting that all neurons responded to J1/tenascin. J1/tn2 shifted the distribution of neurite lengths on substrate-bound J1/tenascin to control values, whereas J1/tn1 did not significantly alter it. Although routinely used at concentrations of 60 μg/ml, J1/tn2 still strongly interfered with the stimulatory properties of substrate-bound J1/tenascin at 10 μg/ml, whereas J1/tn1 did not show any effects when applied even at concentrations of 250 μg/ml (not shown). Nei-
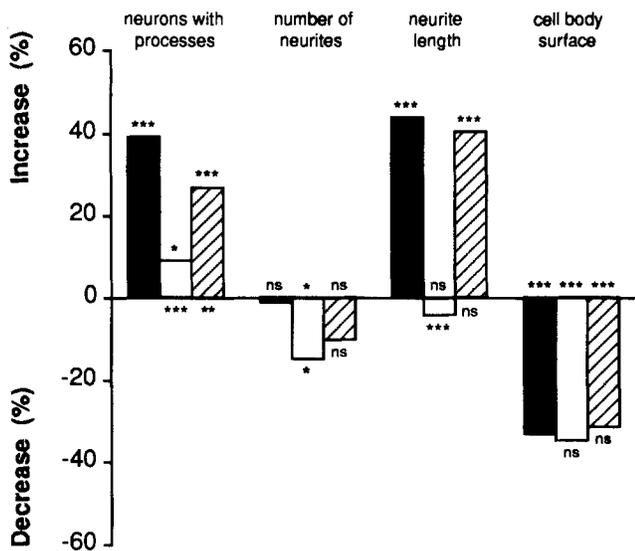
Figure 3. Effect of substrate-bound Jl/tenascin on the morphological differentiation of cultured hippocampal neurons. Hippocampal neurons were maintained on polyornithine-coated plastic (control), or Jl/tenascin adsorbed to polyornithine-coated plastic in the absence (■) or presence of mAbs Jl/tn2 (□) or Jl/tnl (▨). After 24 h control values were for the fraction of neurons with at least one neurite 48 ± 3.2%. Control values for the total number of neurites (187 ± 28.4), total length of neurites (4,795 ± 608 μm), and cell body area (7,635 ± 1,059.1 μm^2) were determined in duplicate for samples of 50 process-bearing neurons per culture well. Columns represent the percent increase or decrease over control values and were calculated as ([parameter$_{test}$-parameter$_{control}$]/parameter$_{control}$) × 100. 500 neurons (two wells with 50 neurons from five independent experiments) were analyzed for each value, except for substrate-bound Jl/tenascin in the presence of monoclonal Jl/tnl antibodies where 300 neurons (two wells with 50 neurons from three independent experiments) were analyzed. Significance levels according to the LSD- and chi-square tests are indicated by the following symbols: (***) P ≤ 0.001; (**) 0.001 < P ≤ 0.01; (*) 0.01 < P ≤ 0.05; (ns) not significant. Symbols above the columns refer to difference from the control and symbols below the columns refer to differences from substrate-bound Jl/tenascin.

ther Jl/tnl nor Jl/tn2 modified neuron attachment to and neurite extension on the control substrate when used at concentrations up to 250 μg/ml (not shown).

Soluble Jl/tenascin Inhibits Neurite Extension

When added to the culture medium 1 h after cell plating on the control substrate, soluble Jl/tenascin retarded neurite extension from hippocampal neurons (Fig. 5). The effect was concentration dependent with a tendency to saturation observable at 15 μg/ml, the amount of Jl/tenascin used for all further experiments (not shown). The proportion of process-bearing cells, total number of neurites and total neurite length were significantly reduced in the presence of soluble Jl/tenascin (Fig. 6). A cumulative frequency distribution plot indicates that neurite lengths were evenly shifted to lower values (not shown). Jl/tnl and Jl/tn2 did not neutralize the inhibitory properties of soluble Jl/tenascin on neurite extension when used at 60 or 250 μg/ml. In contrast, when Jl/tenascin was boiled for 10 min, the inhibitory properties of soluble Jl/tenascin were largely abolished (Fig. 5 C and not shown).
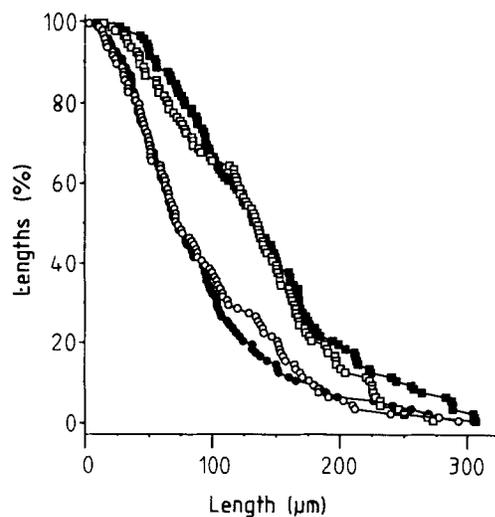

Figure 4. Effect of substrate-bound Jl/tenascin on neurite lengths of cultured hippocampal neurons. Cumulative frequency distribution plot of the percentage of hippocampal neurons (ordinate) with neurites longer than a given length in μm (abscissa) on the control substrate (●) and substrate-bound Jl/tenascin in the absence (■) or presence of mAbs Jl/tnl (□) or Jl/tn2 (o). Individual values are from 100 neurons in two wells for each condition taken from one representative experiment.

Inhibition of neurite outgrowth by soluble Jl/tenascin could not be detected within the first 12 h after cell plating on the polyornithine substrate. 0.5, 1.5, 4.5, and 12 h after plating 5 ± 0.5, 6 ± 0.7, 7 ± 1.4, and 36 ± 3.8% of neurons, respectively, had developed neurites in the absence of soluble Jl/tenascin, while 1.5, 4.5, and 12 h after plating 8 ± 1.7, 12 ± 2.0, and 34 ± 7.4% of neurons, respectively, had developed processes on polyornithine when soluble Jl/tenascin was added to the cultures 1 h after plating. It is noteworthy that a lag phase of at least 4.5 h in neurite outgrowth on polyornithine in the absence or presence of soluble Jl/tenascin could not be detected on substrate-bound Jl/tenascin where 0.5, 1.5, 4.5, and 12 h after cell plating 14 ± 1.7, 26 ± 1.7, 38 ± 2.3, and 68 ± 3.0% of neurons, respectively, had grown out neurites. Each value was derived from 300 neurons of two independent experiments.

To exclude that the effects of Jl/tenascin were because of preferential substrate adherence of a subclass of hippocampal neurons under different culture conditions, the following experiment was carried out: hippocampal neurons were maintained for 24 h on the control substrate in the absence or presence of soluble Jl/tenascin and on substrate-bound Jl/tenascin. Cells were then gently removed from these substrates and further cultured for 24 h by switching substrates (Table I). The effects of substrate-bound and soluble Jl/tenascin appeared completely reversible in that neurons first grown on substrate-bound Jl/tenascin and then subcultured on the control substrate, on substrate-bound Jl/tenascin or in the presence of soluble Jl/tenascin responded in the same manner as freshly dissociated cells. Similar results were obtained when the cells were first cultured under control conditions or in the presence of soluble Jl/tenascin (Table I).

The inhibitory properties of soluble Jl/tenascin were not dependent on the culture substrate. When hippocampal neurons were grown on substrate-bound laminin, fibronectin, or
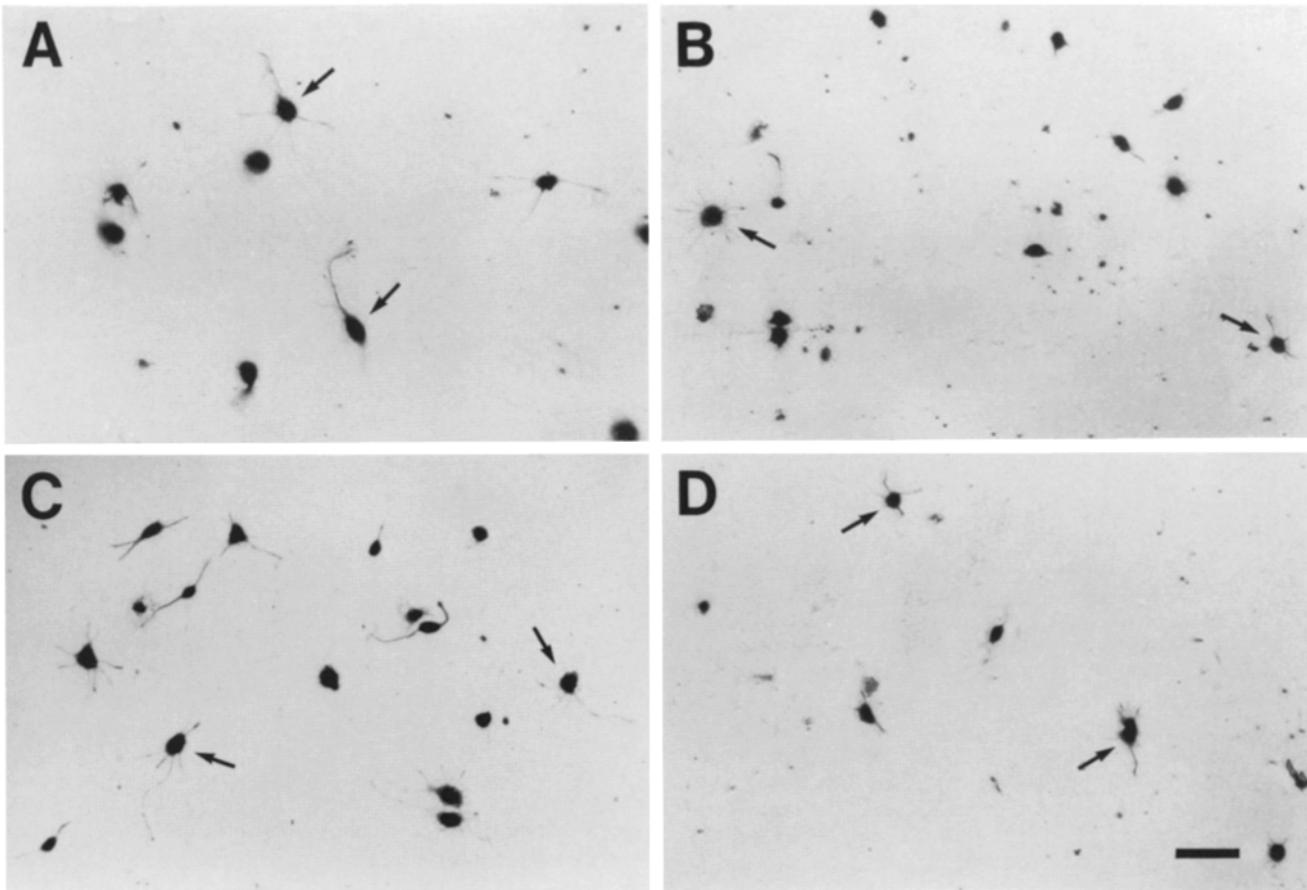
Figure 5. Morphology of hippocampal neurons cultured in the presence of soluble Jl/tenascin. Bright-field micrograph of hippocampal neurons cultured for 24 h on polyornithine (*A*), control or on polyornithine in the presence of soluble Jl/tenascin (*B*), heat-inactivated soluble Jl/tenascin (*C*), or soluble Jl/tenascin in the presence of monoclonal Jl/tn2 antibodies (*D*). Cells were stained with toluidine blue. Typical process-bearing neurons are marked by arrows. Note the retarded development of process-bearing neurons in *B* and *D* as compared to *A* and *C*. Bar, 20 μm.
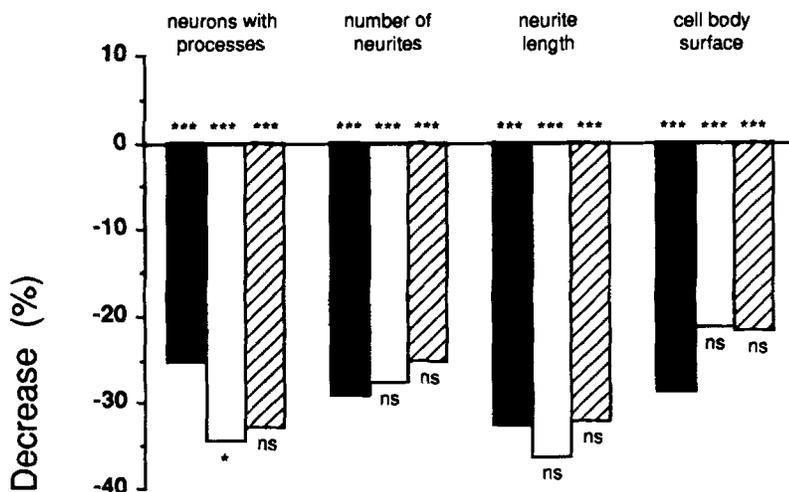

Figure 6. Effect of soluble Jl/tenascin on the morphological differentiation of cultured hippocampal neurons. Hippocampal neurons were maintained on polyornithine (control), on polyornithine-coated plastic in the presence of soluble Jl/tenascin (■), or of soluble Jl/tenascin in the presence of monoclonal Jl/tn2 (□) or Jl/tnl (▨) antibodies. After 24 h, control values were for the fraction of neurons with at least one neurite 52 ± 5.8%. Control values for the total number of neurites (196 ± 17.6), total length of neurites (4,890 ± 687.5 μm), and cell body area (7,279 ± 770.3 μm^2) were determined for samples of 50 process-bearing neurons per culture well. Columns represent the percent increase or decrease over control values and were calculated as ([parameter$_{test}$ − parameter$_{control}$]/parameter$_{control}$) × 100. 400 neurons (four independent experiments) were analyzed for each condition. Significance levels according to the LSD- and chi-square tests are indicated by the following symbols: (***) $P \leqslant 0.001$; (**) $0.001 < P \leqslant 0.01$; (*) $0.01 < P \leqslant 0.05$; (*ns*) not significant. Symbols above the columns refer to differences from the control and symbols below the columns refer to differences from soluble Jl/tenascin in the absence of antibodies.

Table I. Effect of Soluble and Substrate-bound J1/tenascin on Neurite Outgrowth from Cultured Hippocampal Neurons on Switched Substrates

Condition for the second 24 h	Condition for the first 24 h		
	Control	bTN	sTN
Control	4,665	4,295	4,870
bTN	6,040 ***	5,475 ***	6,180 ***
sTN	2,645 ***	2,230 ***	2,890 ***

Hippocampal neurons were maintained for 24 h under three different culture conditions as indicated. Cells were then trypsinized and maintained for another 24 h on switched substrates as indicated. Total neurite lengths of 50 neurons from one representative experiment are shown. Significance levels indicated in each column are related to differences from the control value of the respective column. Significance levels according to the chi-square test are indicated by ***, P ≤ 0.001. (bTN) Substrate-bound J1/tenascin; (sTN) soluble J1/tenascin.

J1/tenascin (preparations are shown in Fig. 1), addition of soluble J1/tenascin resulted in a decrease of total neurite length in all cases (Table II). Furthermore, neurons dissociated from mesencephalon and striatum showed similar responses to soluble and substrate-bound J1/tenascin as hippocampal neurons (not shown).

Substrate-bound and Soluble J1/tenascin Inhibit Spreading of Neuronal Cell Bodies and Decrease Neuron-Substrate Adhesion

The spreading of neuronal cell bodies was reduced by ~30% on substrate-bound J1/tenascin or in the presence of soluble J1/tenascin (Figs. 3 and 6). To further investigate this phenomenon and to examine whether a correlation exists between spreading of neuronal cell bodies and their ability to

Table II. Effect of Soluble J1/tenascin on Neurite Lengths of Cultured Hippocampal Neurons on Substrate-bound J1/tenascin, Laminin, and Fibronectin

Condition	Total neurite length of 50 neurons in μm ± SD	Significance	
		Compared to control	Compared to ECM
Control	2,794 ± 234.1		
bTN	5,850 ± 208.7	*** (***)	
			to bTN
bTN + sTN	3,495 ± 139.6	* (***)	*** (***)
bLN	5,280 ± 494.9	*** (***)	
			to bLN
bLN + sTN	3,308 ± 188.8	NS (*)	*** (***)
bFN	4,104 ± 181.8	*** (***)	
			to bFN
bFN + sTN	3,308 ± 188.8	NS (NS)	* (***)

Hippocampal neurons were maintained for 24 h on different substrates in the absence or presence of soluble J1/tenascin as indicated in the first column. Total neurite lengths of 50 neurons from two culture wells each were determined in three independent experiments. Significance levels according to the LSD- or chi-square tests are indicated, with the results of the latter put in parentheses, and were related to differences to the polyornithine control substrate or, in cases where soluble J1/tenascin was present, to the ECM-substrate in the absence of soluble J1/tenascin. Significance levels are indicated by the following symbols: (***), P ≤ 0.001; (**), 0.001 < P ≤ 0.01; (*) 0.01 < P ≤ 0.05. (bTN) Substrate-bound J1/tenascin; (bTN + sTN) substrate-bound J1/tenascin in the presence of soluble J1/tenascin; (bLN) substrate-bound laminin; (bLN + sTN) substrate-bound laminin in the presence of soluble J1/tenascin; (bFN) substrate-bound fibronectin; (bFN + sTN) substrate-bound fibronectin in the presence of soluble J1/tenascin; (ECM) extracellular matrix substrate.
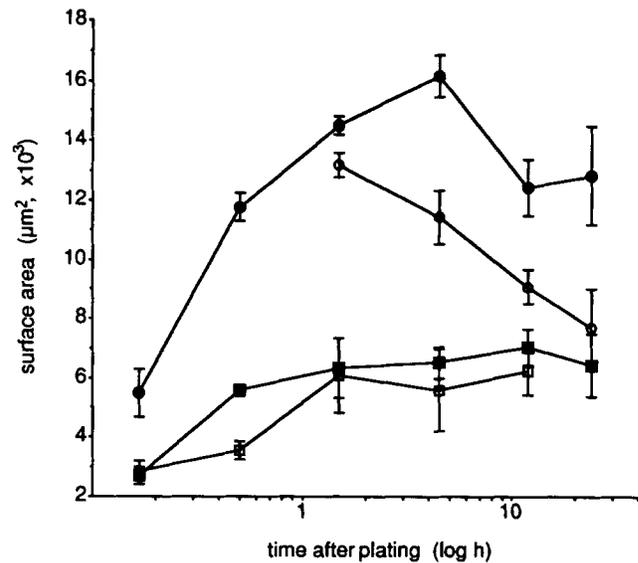

Figure 7. Time course of cell body spreading in the presence and absence of J1/tenascin. Hippocampal neurons were maintained on polyornithine in the absence (control, ●) or presence of soluble J1/tenascin added during (□) or 1 h after (○) cell plating, or on substrate-bound J1/tenascin (■). Mean values of cell body surface areas (ordinate) are semilogarithmically plotted as a function of time after cell plating (abscissa). For each value 300 neurons (six wells with 50 neurons from two independent experiments) were analyzed. Mean values ± SD are shown. All values are significantly different from the control according to the LSD- and chi-square tests (P ≤ 0.001).

extend neurites, spreading of cell bodies of hippocampal neurons was investigated under the same conditions as neurite extension. On the polyornithine substrate, the surface area of the cell body increased continuously during the first 4.5 h after plating (Fig. 7). After 12 and 24 h the surface area was decreased by ~20%, when compared to the value at 4.5 h. When J1/tenascin was added to the culture medium 1 h after cell plating on polyornithine, the initial spreading of the cell body seen at 4.5 h was reversed. When the cells were plated on substrate-bound J1/tenascin or on polyornithine in the presence of soluble J1/tenascin cell spreading was strongly impaired at all time points tested (Fig. 7). Because of the inclusion of nonprocess-bearing cells, the overall sur-

Table III. Effect of J1/tenascin on Neuron-Substrate Adhesion

Condition	Number of cells ± SD	
	2 h	24 h
Control	384 ± 42.9	341 ± 43.6
bTN	31 ± 16.2	15 ± 11.0
sTN	41 ± 18.1	27 ± 10.4

Hippocampal neurons were grown in Terasaki dishes on polyornithine-coated plastic (control), substrate-bound J1/tenascin (bTN), or in the presence of J1/tenascin added to the culture medium directly after plating (sTN). After 2 or 24 h of incubation cells were subjected to mechanical forces and the residual neurons attached to the substrate were counted. 18 wells from three independent experiments were analyzed for each condition and one Terasaki dish was used per time point and experiment for all treatments. According to the LSD-test the values obtained for bTN and sTN differ significantly (P ≤ 0.001) from the polyornithine control values.
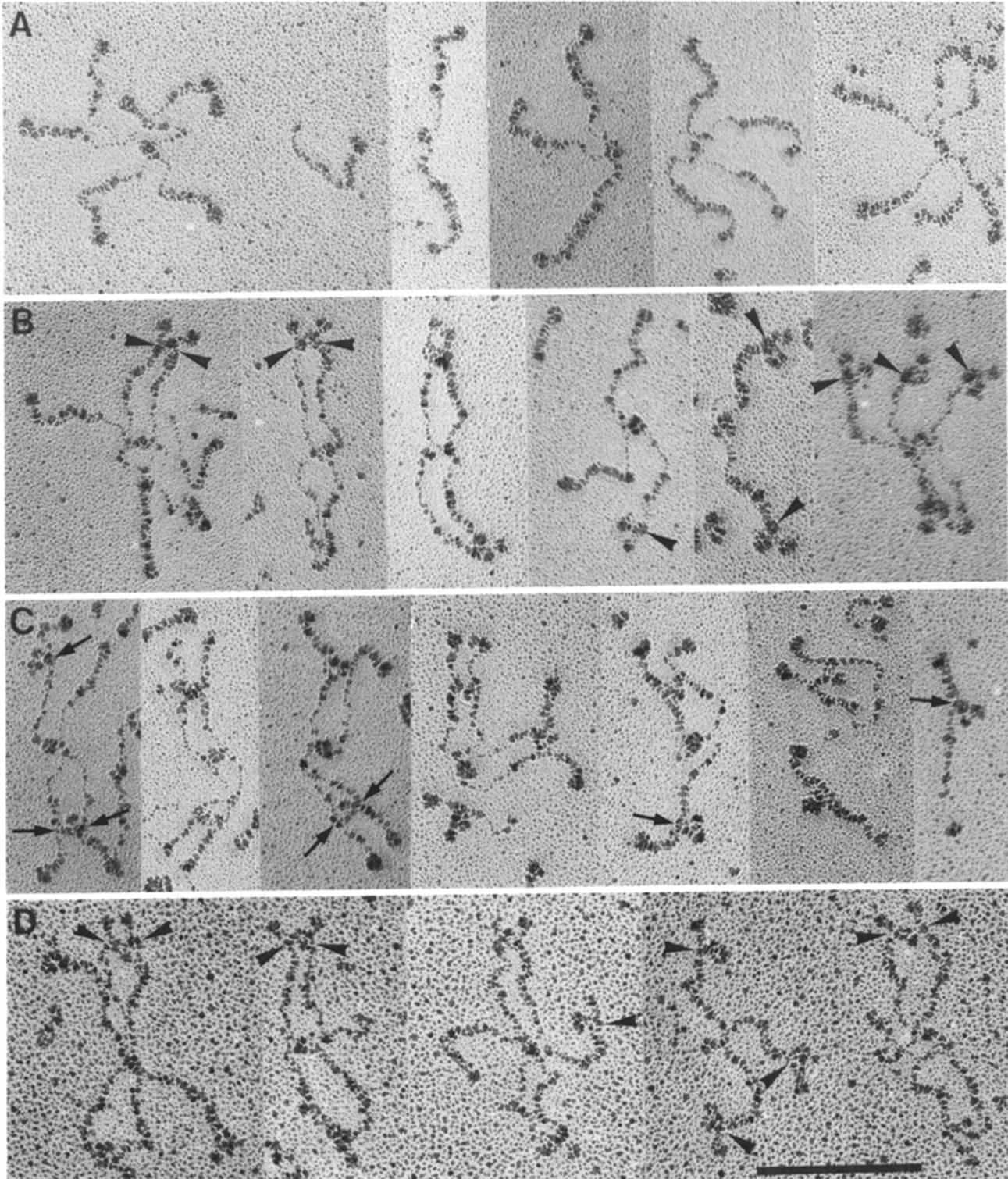
Figure 8. Analysis of antibody binding domains of J1/tenascin molecules by rotary shadowing. J1/tenascin from mouse brain (*A*, *B*, and *C*) or human glioma (*D*) was incubated with mAbs J1/tn2 (*B* and *D*) or J1/tn1 (*C*). In *A* a variety of typical oligomers extracted and purified from mouse brains is shown. In *B* and *D* the J1/tn2 epitope is indicated by arrowheads and in *C* that of J1/tn1 by arrows. Both antibodies frequently cross-linked adjacent J1/tenascin polypeptides. Bar, 100 nm.

face covered by the neurons was found increased, as compared to the analysis of neurite-growing cells alone. This might reflect the formation of veil-like lamellipodia by many neurons without processes. To investigate implications of the anti-spreading effect of J1/tenascin its influence on neuronal substrate adhesion was determined. When neurons were plated on substrate-bound J1/tenascin or in the presence of the soluble molecule, the vast majority (~95%) of the
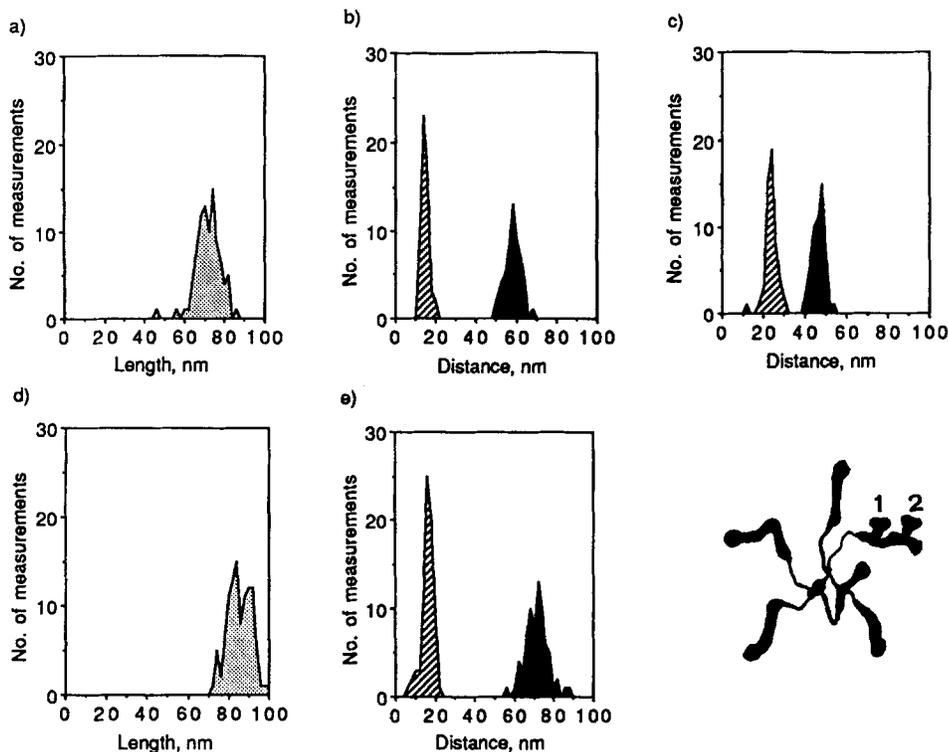
Figure 9. Histograms of antibody binding site distributions. Number of measurements (ordinate) plotted against the curvilinear length of the arms from the T-junction to the center of the distal knob (abscissa) for Jl/tenascin from mouse (*a*) and human (*d*). The position of the Jl/tn1 epitope as measured from the T-junction (*black*) or the distal domain (*hatched*) is shown in *c* for mouse Jl/tenascin. The corresponding position of the Jl/tn2 epitope is shown in *b* and *e* for the mouse and human molecules, respectively. A diagrammatic representation of the epitopes for Jl/tn1 (*1*) and Jl/tn2 (*2*) is shown. Both mouse and human preparations contain a mixture of isoforms, resulting in a broad distribution of values encompassing the variable spliced region of the type III homology domains.

cells could be detached from the bottom of Terasaki wells by mechanical forces which left ~50% of the neurons on the polyornithine control (Table III), indicating a reduction of neuronal substrate attachment forces.

Localization of Antibody Binding Sites on Jl/tenascin

Electron micrographs of rotary shadowed Jl/tenascin from mouse brain revealed monomers and a variety of oligomers ranging from dimers to hexamers (Fig. 8 *A*). The lengths of the arms from the T-junction to the center of the distal knob (fibrinogen homology domain), including the thin proximal portion (EGF-like repeats) and the thickened distal region (fibronectin type III homology repeats) were measured (Fig. 9) and are listed in Table IV. The corresponding lengths for Jl/tenascin from the human glioma cell line U 251 MG are listed for comparison. The difference between mouse and human in the length of the type III homology regions of 10 nm corresponds to a difference of two to four domains. Mouse Jl/tenascin was incubated with Jl/tn1 and both mouse and human Jl/tenascin were incubated with the species cross-

reactive antibody Jl/tn2. Typical examples of Jl/tenascin oligomers labeled with the antibody are shown in Fig. 8. Arms were commonly cross-linked by the divalent antibodies. Both antibodies bound within the thickened distal portion of Jl/tenascin which corresponds to the fibronectin type III homology region (Spring et al., 1989; Kaplony and Vaughan, manuscript submitted for publication). For measurement of the antibody-binding sites, only those which were clearly depicted alongside, but still touching the arm, were chosen. The resultant distributions of Jl/tn1 and Jl/tn2, measured for both the T-junction and the distal end, are shown in Fig. 9. The epitope for the Jl/tn2 antibody is located 57 ± 4 nm (*n* = 53) from the T-junction in mouse and 70 ± 6 nm (*n* = 73) in human Jl/tenascin. The distances of the Jl/tn2 epitope from the distal ends are 14 ± 2 nm (*n* = 53) and 15 ± 3 nm (*n* = 76) in mouse and human, respectively. Subtracting the contributions of the distal knobs leaves in both cases a distance of 12 ± 3 nm into the type III region, measured from the distal end. Antibody Jl/tn1 binds 47 ± 3 nm (*n* = 53) from the T-junction and 23 ± 3 nm (*n* = 53) from the distal end of mouse Jl/tenascin (Figs. 8 and 9).

Table IV. Morphometric Analysis of mAb Binding Sites on Jl/tenascin from Mouse Brain and Human Glioma by EM of Rotary-shadowed Molecules

	Mouse	Human
Whole arm	71 ± 6 nm (*n* = 94)	85 ± 6 nm (*n* = 106)
EGF-repeat region	27 ± 3 nm (*n* = 55)	32 ± 3 nm (*n* = 54)
Type III repeats	43 ± 4 nm (*n* = 55)	53 ± 5 nm (*n* = 54)

The average curvilinear length of the Jl/tenascin arms from the T-junction to the center of the distal knob is shown for mouse and human. The relative distribution of the thin proximal region (EGF-like repeats) and the thickened distal portion (fibronectin type III repeats), which includes the variable spliced region, are listed.

Discussion

Recent investigations have shown that tenascin-like molecules are poor substrates for the culture of dissociated central or peripheral nervous system neurons plated under normal gravity conditions (Friedlander et al., 1988; Chiquet, 1989; Wehrle and Chiquet, 1990; Faissner and Kruse, 1990). When adsorbed to a favorable substrate for neural cells, polyornithine (Collins, 1978), Jl/tenascin shows repulsive properties for the attachment of CNS neuronal cell bodies without, however, inhibiting neurite outgrowth (Faissner and Kruse, 1990). This indicates that the molecule might modu-

late neural cell behavior in an environment conducive for neuronal differentiation. To investigate the influence of J1/tenascin on the establishment of neuronal morphology, we have cultivated single embryonic rat CNS neurons in defined medium at low density on polyornithine-coated plastic surfaces (control), on J1/tenascin adsorbed to the polyornithine substrate (substrate-bound J1/tenascin), and in presence of J1/tenascin added to the culture medium (soluble J1/tenascin). We propose three major inferences from our observations: (a) a molecular domain inducing neurite outgrowth on substrate-bound J1/tenascin involves the fibronectin type III homology repeats; (b) substrate-bound and soluble J1/tenascin affect neurite outgrowth in opposite ways, in that substrate-bound J1/tenascin promotes and soluble J1/tenascin inhibits neurite outgrowth; and (c) spreading of neuronal cell bodies is reduced both by substrate-bound and soluble J1/tenascin, probably by a molecular mechanism not involving the neurite outgrowth promoting domain of the molecule.

J1/tenascin bound to polyornithine is a neurite outgrowth promoting molecule for neurons from hippocampus, mesencephalon, and striatum. This observation parallels recent results showing that tenascin adsorbed to polylysine fosters neurite outgrowth by embryonal chicken sensory, spinal cord, and sympathetic neurons (Wehrle and Chiquet, 1990). The neurite outgrowth promoting effect of J1/tenascin was apparent shortly after cell plating, in contrast to the inhibition of neurite outgrowth by the soluble molecule (see below). The neurite outgrowth promoting property was neutralized by the mAb J1/tn2 but not by the control antibody J1/tn1. This indicates that the J1/tn2 epitope is functionally connected with or identical to a neurite outgrowth–inducing domain of the glycoprotein. Rotary shadowing analysis showed that the J1/tn2 epitope localizes to the fibronectin type III repeats of J1/tenascin. Assuming that a fibronectin type III repeat of M_r 10,000 approximates a sphere with a diameter of 2.9 nm, the epitope for J1/tn2 is on the third or fourth repeat from the distal, carboxyterminal end of the molecule which is marked by the fibrinogen knob. This would place the epitope in either repeat 12 or 13 in the human protein (Gulcher et al., 1989). These repeats are homologous with the fibronectin type III repeats eight and nine of chicken tenascin (Spring et al., 1989). The epitope of the mAb Tn68 that is involved in tenascin-induced hemagglutination of sheep red blood cells and binding of fibroblasts to chicken tenascin (Chiquet-Ehrismann et al., 1988; Spring et al., 1989) is in a region of 104 amino acids within the tenth and eleventh fibronectin type III repeats of chicken tenascin. J1/tenascin from mouse has a similar structural organization and its cDNA sequence displays substantial homology (Weller et al., 1991) to the one reported for chicken tenascin (Spring et al., 1989), cytotactin (Jones et al., 1989), and human hexabrachion (Gulcher et al., 1989). Thus, distinct domains in the fibronectin type III homology repeats seem implicated in cell attachment to and neurite outgrowth on substrate-bound J1/tenascin.

Whereas substrate-bound J1/tenascin promotes extension of neurites, soluble J1/tenascin interferes with neurite outgrowth. In this respect J1/tenascin behaves like soluble cytotactin which inhibits the outgrowth of fiber bundles from embryonic chicken dorsal root ganglion explants at 10 μg/ml, a concentration comparable to the one used in the present study (Crossin et al., 1990). This inhibitory property of J1/tenascin could be observed with hippocampal, mesencephalic, and striatal neurons plated on the polyornithine substrate alone, or on substrate-bound J1/tenascin, laminin, or fibronectin. The opposite effects of J1/tenascin on neurite outgrowth thus parallel results obtained with a neuroblastoma cell line for which substrate-bound tenascin increased and soluble tenascin slowed down the cellular migration rate (Halfter et al., 1989). The inhibitory effect of soluble J1/tenascin was not affected by our mAbs, even when these were used in about 20-fold excess over the ECM constituent on a molar basis. The observation that J1/tn2 interferes with promotion and not with inhibition of neurite outgrowth could indicate differences in the molecular bases for promotion and inhibition of neurite extension by J1/tenascin. This contention is supported by the observation that the mAb Tn68, which recognizes the cell-binding site of tenascin for fibroblasts and binds to a region distinct from the J1/tn2 epitope (see above), inhibits the reduction of neuroblastoma migration rates by soluble tenascin (Halfter et al., 1989). It can at present, however, not be excluded that the inability of J1/tn2 to block the effects of soluble J1/tenascin is a consequence of conformational transitions between the soluble and the substrate-bound form of the glycoprotein. These putative conformational changes might attenuate—or even abolish—the neutralizing effect of J1/tn2 binding on the functional properties of the molecule. Further, the multivalency of J1/tenascin might render it difficult to block its functional regions by antibodies in solution. The resolution of this issue will require the separate preparation of the different molecular domains of the glycoprotein.

The inhibitory effect of soluble J1/tenascin was concentration dependent and sensitive to heat treatment. As control, the antibodies J1/tn1 or J1/tn2 added to neurons growing on polyornithine or substrate-bound laminin and fibronectin in up to 20-fold higher concentration (on a molar basis) than soluble J1/tenascin did not affect neurite growth. The specificity of the dual, opposite effects of J1/tenascin is further supported by recent results obtained with other extracellular matrix components. Laminin and fibronectin, molecules which also show neurite outgrowth promoting properties (for review see Sanes, 1989), have been described to influence the differentiation of mesencephalic neurons in vitro in different ways, depending on their mode of presentation. Soluble laminin and substrate-bound fibronectin induce a strongly polarized neuronal phenotype in which axonal is favored at the expense of dendritic growth. Conversely, soluble fibronectin and substrate-bound laminin enhance expansion of the dendritic compartment as compared to the growth of the axon (Chamak and Prochiantz, 1989; Rousselet et al., 1990). Based on experiments correlating neuronal spreading with adhesion the authors provided evidence that conditions of high adhesion (in case of substrate-bound laminin and soluble fibronectin) support dendritic whereas conditions of low adhesion (in case of soluble laminin and substrate-bound fibronectin) promote axonal growth. Further reduction of adhesion may ultimately lead to inhibition of both axonal and dendritic growth. In this context it is noteworthy that substrate-bound J1/tenascin displays some polarizing activity by lengthening the axonlike longest process by 70%, but the dendritelike shorter processes by only 20%, whereas soluble tenascin inhibits the elongation of both classes of processes to a similar degree (our unpublished observations). How-

ever, since both soluble and substrate-bound J1/tenascin reduce the attachment of neurons to the culture substrate in a comparable manner (see below), the differential effects of the molecule on process outgrowth appear not to be directly correlated with the degree of cell adhesivity.

Soluble J1/tenascin inhibits neurite outgrowth on different substrates. Therefore, it is unlikely that the inhibitory effect of soluble J1/tenascin is because of the direct blocking of the cell surface receptors for laminin, fibronectin, or the substrate-bound form of J1/tenascin. The retardation of neurite outgrowth by soluble J1/tenascin becomes apparent after an incubation period of 12 h. This might indicate that J1/tenascin does not interfere with the initiation, but with the stabilization of neurites, a process known to involve the cytoskeleton (Bray and Hollenbeck, 1988). Tenascin has, indeed, been suggested to interfere with cytoskeletal organization in fibroblasts, suppressing cell spreading on tenascin or mixed tenascin/fibronectin substrates (Chiquet-Ehrismann et al., 1988; Lotz et al., 1989). It is likely that similar mechanisms hold true for neurons also. Explanations for the dichotomy in the effects of soluble versus substrate-bound J1/tenascin cannot be given at present, but it may be related to, although not exclusively caused by differences in presentation of the molecule as soluble versus substrate-bound ligand.

Both soluble and substrate-bound J1/tenascin reduce the spreading of neuronal cell bodies. This is in accordance with the anti-spreading effect of tenascin for fibroblasts, neural crest derivatives and other cell types investigated so far (Tan et al., 1987; Mackie et al., 1988; Chiquet-Ehrismann et al., 1988; Friedlander et al., 1988; Bourdon and Ruoslahti, 1989; Halfter et al., 1989; Riou et al., 1990). The inhibitory influence of J1/tenascin on cell body spreading is probably responsible for the weaker attachment of neurons to the J1/tenascin-coated polyornithine substrate which we observed in the present study. Under conditions of high cell density plating the reduction of cell-substrate adherence leads to the preponderance of cell-cell adhesion forces (Jessel, 1988). The aggregation of neurons and fasciculation of neurites on homogeneous and the preferential enrichment of neurons or neurites on J1/tenascin-free as compared to J1/tenascin-containing areas of patterned J1/tenascin/polyornithine (Faissner and Kruse, 1990) or cytotactin/laminin (Crossin et al., 1990) substrates might hence be because of interference of J1/tenascin with neuron-substrate contact formation. The molecular domain of J1/tenascin responsible for inhibition of spreading appears not related to the J1/tn2-binding site involved in neurite outgrowth since this mAb does neither abolish the anti-spreading nor the repulsive effect of J1/tenascin (this study; and Faissner and Kruse, 1990). It is noteworthy that the antibody Tn68 neutralizes the anti-spreading effect of tenascin on fibroblasts (Chiquet-Ehrismann et al., 1988), underscoring that the antibodies Tn68 and J1/tn2 are functionally distinct and recognize separate sites on the molecule, in accordance with the conclusions drawn from our rotary shadowing studies. Recent investigations with fusion proteins have localized a cell-binding site to the carboxyterminal and an anti-spreading domain to the aminoterminal half of tenascin. The mAb Tn68, which counteracts the anti-spreading effect of the molecule, inhibits the binding of fibroblasts to the cell-binding fragment (Chiquet-Ehrismann et al., 1988, Spring et al., 1989). In this perspective, the interaction of a cell with the cell-binding domain seems a prereq-

uisite for the anti-spreading activity of tenascin. Recent measurements of the adhesive forces developing between cells and tenascin compared with fibronectin have shown a functional dissociation of the initial binding event and cell spreading for fibroblasts (Lotz et al., 1989).

The molecular dichotomy relating to cell body spreading on the one hand and neurite outgrowth on the other hand is remarkable in view of the transducing signals presumably elicited in the J1/tenascin-responsive neurons. Since our study shows that these two mechanisms may be different, the challenging problem emerges of how cellular receptors can effect such compartmentalized communication with the cytoskeleton that directs cell body spreading and neurite outgrowth. Bourdon and Ruoslahti (1989) have reported the isolation of an RGD peptide-dependent integrin by affinity chromatography on a tenascin column. Yet, it is unlikely that RGD peptide-dependent integrins mediate any of the neuronal responses to J1/tenascin, since the GRGDS peptide did not interfere with the neurite outgrowth stimulating or inhibiting properties of J1/tenascin in our assay systems and since tenascin from mouse fibroblasts lacks the RGD motive (Weller et al., 1991). Other receptor candidates have been described for J1/tenascin, including heparin (Marton et al., 1989; Faissner et al., 1990) and a chondroitinsulfate proteoglycan (Hoffman et al., 1987, 1988).

In conclusion, our study suggests that J1/tenascin is a multifunctional molecule which exerts both inhibitory and stimulatory influences on neurite outgrowth depending on whether it is presented to the cell in substrate-bound or soluble form. In our view, it is plausible to assume that these different features involve at least two distinct functional domains, one that promotes neurite outgrowth and another (others) that inhibit(s) neurite outgrowth and/or substrate attachment of neuronal cell bodies. It is tempting to speculate that the multitude of functional properties of J1/tenascin and its corresponding receptor mechanisms might be implicated in such different functional events as the establishment of barrelfield boundaries in the somatosensory cortex (Steindler et al., 1989a,b), patterns of neurite outgrowth in the developing and regenerating peripheral nervous system (Martini and Schachner, 1991; Martini et al., 1990) and directing regrowing axons to the original synaptic sites at the denervated neuromuscular junction (Sanes et al., 1986; Daniloff et al., 1989; Gatchalian et al., 1989). The elucidation of the asserted functional role(s) of J1/tenascin in these events will require the establishment of assay systems designed to reflect the more complex situations in vivo.

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