Hypothalamic perineuronal net assembly is required for sustained diabetes remission induced by fibroblast growth factor 1 in rats

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We recently showed that perineuronal nets (PNNs) enmesh glucoregulatory neurons in the arcuate nucleus (Arc) of the mediobasal hypothalamus (MBH)1, but whether these PNNs play a role in either the pathogenesis of type 2 diabetes (T2D) or its treatment remains unclear. Here we show that PNN abundance within the Arc is markedly reduced in the Zucker diabetic fatty (ZDF) rat model of T2D, compared with normoglycaemic rats, correlating with altered PNN-associated sulfation patterns of chondroitin sulfate glycosaminoglycans in the MBH. Each of these PNN-associated changes is reversed following a single intracerebroventricular (icv) injection of fibroblast growth factor 1 (FGF1) at a dose that induces sustained diabetes remission in male ZDF rats. Combined with previous work localizing this FGF1 effect to the Arc area1,9, our finding that enzymatic digestion of Arc PNNs markedly shortens the duration of diabetes remission following icv FGF1 injection in these animals identifies these extracellular matrix structures as previously unrecognized participants in the mechanism underlying diabetes remission induced by the central action of FGF1.

PNNs are a distinct extracellular matrix subtype that enmesh neurons in defined circuits and interdigitate with peri-synaptic astrocytic processes to influence neurocircuit connectivity and synaptic stability1. We recently reported that, in both humans and rodents, PNNs enmesh a tightly packed cluster of neurons situated at the junction of the Arc and adjacent median eminence (ME) in the MBH1. GABAergic agouti-related peptide (AgRP) neurons are among those that are both enmeshed by PNNs and implicated in the physiological control of food intake and glucose homeostasis2,3. Given the powerful influence that PNNs can exert over the neurons they enmesh, we sought to determine if obesity-associated diabetes is correlated with maladaptive changes in the formation of these Arc PNNs and if so, whether they might be targets for its treatment.

Our original report describing hypothalamic PNN matrices was based primarily on immunofluorescence (IF) labelling of PNN-associated chondroitin sulfate (CS) glycosaminoglycans (GAGs) using Wisteria floribunda agglutinin (WFA) in mice1. Extending this work, we report that in normoglycaemic Wistar rats, (1) Arc PNNs are enriched in the CS proteoglycan (CSPG) aggrecan4, and (2) these PNNs enmesh neurons throughout both medial Arc (ArcM) and lateral Arc/retrochiasmatic (ArcL) regions (Fig. 1a). Comprehensive stereological mapping throughout the MBH reveals that these PNNs effectively circumscribe the Arc–ME junction (Supplementary Fig. 1), as reported in mice1. Although aggrecan is a major constituent of PNNs in most brain areas, several features distinguish Arc PNNs from those located elsewhere. First, whereas PNNs in visual and motor cortices tightly enmesh both the neuronal soma and proximal axonal and dendritic processes (Supplementary Fig. 2 and Supplementary Videos 1 and 2), PNNs in the Arc enmesh the neuron soma more loosely and show minimal extensions surrounding proximal processes (Fig. 1b and Supplementary Video 3). Second, while PNNs in the cerebral cortex contain two extracellular PNN glycoproteins that enhance structural stability—Tenascin-R (TnR) and the hyaluronan and proteoglycan link protein 1 (HAPLN-1)—only TnR is present in Arc PNNs (Supplementary Fig. 3). This difference may explain the comparatively diffuse appearance of Arc PNNs compared to cortical PNNs, morphological differences that were also observed in human brain (Supplementary Fig. 4).

To determine if hypothalamic PNN abundance and/or composition differ between diabetic rats and normoglycaemic controls, we performed stereological mapping of PNNs throughout the rostrocaudal extent of the MBH in ZDF rats, an established model of T2D. Compared to that in normoglycaemic Wistar controls (Fig. 1c), the mean fluorescence intensity (MFI) of both WFA and aggrecan were markedly reduced throughout the ArcM and ArcL areas of ZDF rats (Fig. 1d–g and Supplementary Fig. 5). In contrast, no such loss of PNN matrices was observed in visual or motor cortices of these same animals (Supplementary Fig. 6 and Supplementary Videos 4 and 5), suggesting that reduced PNN formation in these animals is specific to the Arc. Further stereological mapping of Arc PNNs in ZDF rats using higher magnification and increased laser imaging power revealed the presence of aggrecan5 PNN matrices in the ArcM region, albeit at markedly reduced levels and more diffuse
in appearance than in normoglycaemic controls (Supplementary Fig. 7 and Supplementary Video 6), whereas PNN-associated WFA immunoreactivity remained undetectable. In addition, loss of aggrecan\(^a\) PNN structures appeared to be somewhat more pronounced in the ArcL than the ArcM throughout its rostrocaudal extent. In summary, (1) ZDF rats, compared with normoglycaemic Wistar rats, exhibit stunted Arc PNN content and assembly, and (2) the loss of PNN-associated WFA staining exceeds loss of the underlying aggrecan core protein. Because ZDF and Wistar rats are on different genetic backgrounds, we also analysed Arc PNNs in non-diabetic Zucker diabetic lean (ZDL) rats, which are derived on the same background as ZDF rats. We report that in these animals, Arc PNNs throughout the ArcM and ArcL areas of the MBH in a and d, scale bar: 200 μm. Higher-magnification orthogonal views of WFA\(^a\) aggrecan\(^a\) PNN enmeshments in b and e, scale bar: 25 μm. Tiled panoramic images of aggrecan from a coronal section (c) from a normoglycaemic Wistar control (left) and an age-matched T2D-ZDF rat (right) shows selective loss of PNN labelling in the Arc of ZDF rats; scale bar: 1 mm. f, g, Quantification of the MFI of PNNs averaged from medial hypothalamic sections (−2.2 to −2.8 mm posterior from bregma) for ArcM (f) and ArcL (g) areas from normoglycaemic Wistar and T2D-ZDF rats, normalized to Wistar MFI averages (nMFI; n = 8 rats per group; mean ± s.e.m.). Data were analysed using a Student’s t-test (unpaired, two-sided). \(P < 0.001\) and \(P < 0.0001\) versus Wistar controls; WFA-ArcM, \(P = 0.0036\); aggrecan-ArcM, \(P = 0.0001\); WFA-ArcL, \(P = 0.0004\); aggrecan-ArcL, \(P = 0.0001\) (f). Two separate cohorts of T2D-ZDF and normoglycaemic Wistar rats were analysed, and the results were reproducible between studies. 3V, third ventricle.
As leptin is implicated in the postnatal development of both Arc neurocircuits and Arc PNNs, and since deficient leptin signalling drives the obese, diabetic phenotype of ZDF rats (owing to a leptin receptor mutation), we next sought to determine if the PNN abnormalities we detected in ZDF rats are specific to this animal model or instead are only found in rats that are hyperglycaemic. Therefore, the MBH of ZDF rat identifies each isomer using specific channels. The release of hyaluronan (HA) by ChABC is also observed in the ΔOS-CS channel. T2D-ZDF rats exhibit changes in the relative percentages of Δ4S-, Δ6S- and Δ2S6S-CS that alter the hypothalamic CS/DS-GAG sulfation patterns and the 4S/6S CS ratio compared to those in age-matched, normoglycaemic Wistar controls (n = 8 rats per group; mean ± s.e.m.). Data were analysed using a Student’s t-test (unpaired, two-sided); ****P < 0.0001 versus Wistar controls. Δ4S-CS, P ≤ 0.0001; Δ6S-CS, P ≤ 0.0001; and Δ2S6S-CS, P ≤ 0.0001 (d).

Fig. 2 | T2D-ZDF rats exhibit abnormal hypothalamic CS/DS-GAG sulfation patterns. a, Schematic of CS and DS sulfation positions. During biosynthesis, repeating CS disaccharide units can be nonsulfated (0S-S), monosulfated (4S-CS and 6S-CS), disulfated (2S6S-CS and 4S6S-CS) or epimerized and disulfated to produce DS (2S4S-DS). b, The proportion of non-, mono- and disulfated isomers influences the function of the underlying extracellular/PNN matrices. c-e, Isolation of CS/DS disaccharides from hyperglycaemic ZDF and normoglycaemic Wistar MBH tissue by ChABC, quantitatively analysed by LC–MS coupled with MRM analysis. Representative MRM analysis of MBH CS/DS disaccharides from a normoglycaemic Wistar rat identifies each isomer using specific channels. The release of hyaluronan (ΔHA) by ChABC is also observed in the ΔOS-CS channel. T2D-ZDF rats exhibit changes in the relative percentages of Δ4S-, Δ6S- and Δ2S6S-CS that alter the hypothalamic CS/DS-GAG sulfation patterns and the 4S/6S CS ratio compared to those in age-matched, normoglycaemic Wistar controls (n = 8 rats per group; mean ± s.e.m.). Data were analysed using a Student’s t-test (unpaired, two-sided); ****P < 0.0001 versus Wistar controls. Δ4S-CS, P ≤ 0.0001; Δ6S-CS, P ≤ 0.0001; and Δ2S6S-CS, P ≤ 0.0001 (d).

In ZDF rats, sustained diabetes remission can be induced by a single icv injection of FGF1 (refs. 2–4) via an action localized to the Arc–ME area, but the mechanisms underlying this response are unknown. To determine if this FGF1 effect is accompanied by reversal of aberrant PNN architecture, we quantified Arc PNN abundance and composition in cohorts of ZDF rats before (aged 5 weeks) and after (aged 19 weeks) the onset of hyperglycaemia (Supplementary Fig. 9a). Here we report that, while PNN-associated CS-GAG expression (detected by WFA staining) was reduced in both the ArcM and ArcL of the older, diabetic cohort of ZDF rats compared to the younger, prediabetic cohort (Supplementary Fig. 9b–d), no differences of either aggrecan expression or MBH CS/DS-GAG sulfation patterns were detected between the two groups (Supplementary Fig. 9e). Together, these findings indicate that while loss of CS-GAG abundance from Arc PNNs is linked to the diabetes phenotype in both ZDF and Wistar rat models of T2D, reduced aggrecan content and aberrant CS/DS-GAG sulfation patterns appear to be intrinsic to the PNNs of ZDF rats and are present before diabetes onset.
transiently reduced following icv FGF1 injection and returned to baseline levels after 8 d, whereas intake remained unchanged in ad libitum-fed, icv vehicle-treated controls.

The effect of icv FGF1 injection, compared with injection of vehicle controls, to induce sustained blood glucose lowering was associated with significant increases of both ArcM and ArcL PNN content...
and matrix assembly (Fig. 3b–g). By comparison, neither WFA nor aggrecan staining were altered in the adjacent ventromedial nucleus (VMN; Fig. 3h), suggesting that the stimulatory effect of FGF1 on PNN assembly is restricted to the Arc region. Furthermore, aberrant hypothalamic CS/DS-GAG sulfation patterns previously observed in hyperglycaemic ZDF rats (Fig. 2) were also reversed by icv FGF1 injection, as ∆4S-CS isomer content decreased whereas both the ∆6S- and ∆2S6S-CS isomers increased, again with no change of either ∆6S-CS, ∆3S6S-CS or DS (Fig. 3i), thereby normalizing the 4S/6S CS ratio (Fig. 3j). Thus, both the reduced abundance of PNNs and associated alteration of CS/DS-GAG sulfation patterns observed in the MBH of untreated ZDF rats are effectively reversed in association with normalization of glycaemia induced by icv FGF1 injection. To assess both the rapidity with which this FGF1 effect occurs and the extent to which it depends on reversal of hyperglycaemia, we measured Arc PNN abundance 24 h after icv injection of FGF1 (3 μg) or vehicle in nondiabetic Wistar rats. Our finding that a robust increase of Arc PNN abundance was detected within 24 h of icv FGF1 injection in these animals (Extended Data Fig. 2) indicates that both FGF1-induced PNN formation is induced rapidly and that this induction is not dependent on normalization of glycaemia, since blood glucose levels were not affected.

To test the hypothesis that the action of FGF1 on Arc PNNs contributes to the mechanism underlying sustained glucose lowering, we used a well-established method for experimentally induced PNN degradation via targeted microinjection of chondroitinase ABC (ChABC), a bacterial enzyme that selectively cleaves CS/DS-GAGs into their disaccharide units while leaving other sulfated GAG species (for example, heparan sulfate) intact16. Since ChABC also releases aggrecan core proteins from the cell surface, it induces both protein and glycan PNN disassembly17. To verify that hypothalamic PNN digestion is induced by ChABC microinjection in vivo, we performed a unilateral microinjection of ChABC targeting the MBH of Wistar rats (17.5 mU in a volume of 175 nl) and compared PNN content between the injected and uninjected sides 4 d later. As expected, WFA* labelling of CS-GAGs was absent, and stable aggrecan* PNN structures were reduced from the Arc on the side receiving ChABC, whereas PNNs remained intact on the uninjected side (Supplementary Fig. 10a). PNN loss was accompanied by a dramatic increase of BE-123 staining of CS 'stubs' (CS neoepitope cleavage products produced on cleaved CS/DS disaccharides only after ChABC-specific digestion of CS/DS-GAGs18), which serves as a biomarker of ChABC activity. Based on the distribution of CS-stub labelling, we also showed that following a single bilateral microinjection, ChABC activity was spread throughout the full rostrocaudal extent of the Arc–ME junction in ZDF rats (Supplementary Fig. 10b).

To test whether the action of FGF1 on hypothalamic PNN assembly is required for the sustained antidiabetic effect of central FGF1 treatment, we developed and implemented a tri-injection surgical procedure in which age- and glycaemia-matched ZDF rats received a single icv injection of FGF1 (3 μg) and during the same procedure underwent bilateral microinjection of either active ChABC (to disassemble PNNs) or vehicle (heat-inactivated ChABC protein) directly to the Arc. Our finding that the FGF1-induced acute-phase (days 1–8) anorexic response and associated weight loss did not differ between groups (area under the curve (AUC) FI1–8, P ≥ 0.6) indicates that the mechanism driving this anorexia is not dependent on intact Arc PNNs (Fig. 4a–c). Because our previous work shows that the initial phase of FGF1-induced glucose lowering in ZDF rats is driven largely by this anorexic response (since pair-fed controls show comparable lowering of blood glucose over the first 8–10 d)19, we anticipated that the initial phase of glucose lowering would not differ significantly between the two groups and this proved to be the case (Fig. 4c; AUCg1–4, grey, P ≥ 0.5). However, once food intake returned to near baseline
levels, blood glucose levels began to diverge between treatment groups. Specifically, blood glucose levels increased sharply towards their baseline level of hyperglycaemia during the post-acute phase (days 10–28) in ZDF rats receiving active ChABC treatment in conjunction with icv FGF1, whereas FGF1-treated animals receiving the heat-inactivated protein control remained normoglycaemic throughout the 28-d study period (Fig. 4c, AUC_{ICG10–28} \( P = 0.009 \)). These findings indicate that intact Arc PNNs are required for the sustained, food intake-independent, antidiabetic action of FGF1, but not the acute, transient reduction of intake or glycaemia.

At the conclusion of the study, we found that among ZDF rats in which icv FGF1 injection was paired with intrahypothalamic microinjection of heat-inactivated protein vehicle, Arc PNN structures were normal in appearance. In contrast, hyperglycaemia relapse in animals receiving icv FGF1 combined with active ChABC treatment was associated with loss of Arc PNN assembly resembling that of naïve ZDF rats (Supplementary Fig. 1). Extending this finding, reductions in both aggrecan and MFI (Fig. 4d) and the number of aggrecan PNN structures (Fig. 4e) were more pronounced in the ArcL than ArcM of these animals. Similarly, we show that, in a small cohort of ZDF rats that received icv FGF1 in combination with unilateral ChABC microinjection, ArcL, but not ArcM, PNNs were markedly reduced on the microinjected side, whereas FGF1-induced PNN formation remained intact on the contralateral, non-injected side (Supplementary Fig. 12). Complementing ChABC-induced loss of stable PNN complexes in the MBH was the appearance of CS ‘stubs’, indicative of successful ChABC targeting.

Taken together, our results show that (1) PNNs are markedly abnormal in both abundance and composition in the Arc of ZDF rats, but not in other brain areas, (2) central administration of FGF1 at a dose that induces sustained diabetes remission corrects these PNN abnormalities and (3) ChABC-mediated disruption of these PNNs markedly shortens the duration of FGF1-induced diabetes remission. We conclude that ZDF rats are characterized by anomalous PNN assembly specifically in the Arc, and that reversal of this defect is required for the sustained antidiabetic action of FGF1 in these animals. These findings constitute direct evidence linking PNN abnormalities and (3) ChABC-mediated disruption of these PNNs to the control of glucose homeostasis and identifies these PNNs as new potential targets for diabetes treatment.

The nature of the PNN abnormality detected in the MBH of ZDF rats warrants comment. This defect is characterized by reduced labelling intensity of PNN structures, rather than their complete absence, which resembles the pattern seen in neuropsychiatric diseases such as schizophrenia and stroke. As PNN loss in the former disease states associates with impaired synaptic connectivity, disruption of inhibitory neural networks and aberrant synaptic refinement, a priority for future work is to determine how anomalous PNN assembly impacts Arc neurocircuits in diabetic animals.

Loss of Arc PNNs in ZDF rats was also accompanied by decreased hypothalamic content of the Δ2565-CS isomer, which enhances growth factor binding and promotes neurite outgrowth, along with an increase in Δ4S-CS, a decrease in Δ6S-CS and a corresponding increase in the 4S/6S CS ratio. As these alterations were present in prediabetic 5-week-old rats, as well as in older, diabetic animals, and since they were not detected in the genetically normal HFD/STZ Wistar rat model of T2D, we suspect that aberrant CS/DS-GAG sulfation patterns observed in the MBH of ZDF rats are manifestations of deficient leptin signalling and are not linked to hyperglycaemia per se. This interpretation is consistent with recent evidence that postnatal maturation of Arc PNNs is dependent on an intact leptin signal. Nevertheless, because this constellation of changes is predicted to both increase matrix stiffness and adversely impact neurocircuit formation, these PNN alterations could potentially impact hypothalamic glucoregulatory neurocircuit development in ways that predispose to hyperglycaemia in the adult. Since these compositional changes are reversed by FGF1, studies to elucidate their role in sustained FGF1-induced blood glucose normalization are warranted.

AgRP neurons are prominent among neurons enmeshed by PNNs in the ArcM, and excessive AgRP neuron activation is a feature of virtually all rodent diabetes models. As reduced melanocortin signalling (which results from AgRP neuron activation) is linked to T2D pathogenesis in humans as well as rodents, it is possible that restored PNN assembly surrounding AgRP neurons contributes to the sustained antidiabetic action of FGF1 by increasing melanocortin signalling. Consistent with this notion is evidence that in mice with diabetes secondary to reduced central melanocortin signalling (Mc4r+/− mice), the duration of diabetes remission induced by icv injection of FGF1 is markedly shortened despite the induction of the expected anorexia, which closely resembles the impact of Arc PNN digestion on the duration of FGF1-induced glucose lowering in ZDF rats. This evidence that Arc PNNs and melanocortin signalling must both be intact for sustained (but not acute) FGF1-induced glucose lowering supports a model in which AgRP neuron activity is constrained by the PNNs that enmesh them, and that loss of these PNNs predisposes to hyperglycaemia by reducing melanocortin signalling.

Whereas AgRP neurons are confined largely to the ArcM, prominent effects of both diabetes and FGF1 on PNNs were observed in the adjacent ArcL region. Specifically, PNN loss in ZDF rat hypothalamus is, if anything, more pronounced in the ArcL than the ArcM region, and ChABC-induced disassembly of PNNs in the hypothalamus of ZDF rats receiving icv FGF1 injection was similarly more prominent in the ArcL than the ArcM. As this PNN disassembly dramatically shortened the duration of FGF1-induced blood glucose lowering, PNN-enmeshed neurons in ArcL emerge as attractive potential targets involved in this effect. Although the identity of these neurons awaits further study, non-AgRP GABAergic neurons are concentrated in the ArcL, and in mice, most of these GABAergic neurons are enmeshed by PNNs. Moreover, a role for these ArcL non-AgRP GABAergic neurons in leptin modulation of energy homeostasis has been suggested. Future studies are warranted to further characterize distinct subsets of PNN-enmeshed neurons in the Arc and ascertain their contributions to glucose homeostasis, diabetes pathogenesis and FGF1 action.

**Methods**

**Study animals.** For baseline characterization of hypothalamic PNNs, naïve male Wistar (Hsd:Wl, Envigo) and male ZDF rats (ZDF-Lepr+/+; Crl, 370 obese, Charles River) were group-housed until 5 weeks or 19 weeks of age in a temperature-controlled room under a 12h:12h light-dark cycle with ad lib access to Purina 5008 diet (Animal Specialties) and water. For PNN identification in naïve male ZDL rats (ZDF-Lepr+/+; 371 lean, Charles River), rats arrived separately and were monitored in parallel housing conditions. For icv FGF1 injection studies, male ZDF rats (aged 6–8 weeks) were cannulated before the onset of hyperglycaemia (Surgery) and allowed to recover for 7 d before injection of either FGF1 (3µg) or saline vehicle into the LV immediately following hyperglycaemia onset (Criteria for diabetes). For studies in which icv FGF1 treatment was coupled with bilateral microinjection of ChABC targeting the MBH, male ZDF rats arrived at 8 weeks of age and again were monitored until the onset of diabetes and were randomly assigned to one of the various treatment groups (detailed below). For the HFD/STZ rat model of T2D, male Wistar rats (aged 8 weeks) were placed on a HFD before induction of stable, moderate hyperglycaemia by administering low-dose STZ (STZ/HFD-Wistar rat model of T2D). All procedures were performed in accordance with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee at the University of Washington, and all experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals. All experiments reported are in compliance with the ARRIVE guidelines (Reporting Summary).

**Criteria for diabetes and diabetes remission.** Although ZDF rats tend to develop diabetes between 8–10 weeks of age, the degree of hyperglycaemia at diabetes onset is variable. In the current studies, diabetes onset was defined as morning blood glucose values &gt; 250 mg dl−1 lasting for at least three consecutive days, and icv...
injections were consistently given within 5 d of diabetes onset. To ensure that we captured both acute- (anorexia-induced) and post-acute phases of FGFI-induced glucose lowering, morning blood glucose levels were obtained under ad lib-feeding conditions at least four times weekly for a minimum of 24 d following icv FGFI injection in ZDF rats. Blood glucose levels were measured by glucometer (NovaMax Plus meter) using the tail prick method.

Surgery. All surgeries were performed under isoflurane anaesthesia using the following stereotaxic coordinates for procedures in ZDF rats: LV cannulation (26-ga, Plastics One): −0.8 mm posterior to bregma, 1.5 mm lateral and −2.6 mm below the skull surface; injection of FGFI or vehicle into the LV under anaesthesia in animals without an indwelling ventricular cannula: −0.7 mm posterior to bregma, 1.8 mm lateral and −3.8 mm below the skull surface; MBH bilateral microinjection of ChABC (or heat-inactivated ChABC protein control): −2.6 mm posterior to bregma, −0.6 mm lateral and −9.8 mm below the skull surface. For unilateral microinjection into the MBH of Wistar rats: −2.8 mm posterior to bregma, 0.4 mm lateral and −10.3 mm below the skull surface. Animals received buprenorphine hydrochloride for postoperative pain control (Reckitt Benckiser).

Intracerebroventricular injections. Rats were monitored for several days to ensure that mean blood glucose values were matched between study groups and fit the criteria for diabetes before the animals received the icv injection. For icv injections via the LV cannula in rats, saline control or recombinant mouse FGF1 (mFGF1; Prospec-Tany TechnoGene), dissolved in sterile water at a concentration of 1.5 μg μl⁻¹, were injected over 60 s in a final volume of 2 μl using a 33-gauge needle extending 0.8 mm beyond the tip of the ivc cannula.

Unilateral and bilateral intraparenchymal microinjections targeting the MBH. After monitoring for several days to ensure that mean blood glucose values were matched between groups and criteria for diabetes were met, rats underwent bilateral and unilateral intraparenchymal microinjection of either active ChABC (17.5 μl; C3667; Sigma-Aldrich) dissolved in artificial cerebrospinal fluid (aCSF) at a concentration of 10 U/100 μl or heat-inactivated ChABC protein vehicle control (17.5 μl) in a volume of 175 nl. Heat inactivation was performed at 85 °C for 45 min, and activity loss was confirmed by in vivo digestion assays. Injections were given at a rate of 1 nl/s using the Nanoject III microinjection system (Drummond Scientific) with autoclaved glass-pulled micropipettes. Enzyme concentration and volume were optimized to ensure spread throughout the rostrocaudal extent of the MBH.

STZ/HFD-Wistar rat model of T2D. After consuming a 60% HFD (D12492, Research Diets) for 2 weeks, Wistar rats received either a single s.c. injection of STZ (Sigma-Aldrich) at a low dose (30 mg kg⁻¹ body weight in a volume of 250 μl) to induce stable, moderate hyperglycaemia (~300–400 mg dl⁻¹). Nondiabetic Wistar control rats were maintained on a standard chow diet and received s.c. injections of 0.02% sodium azide at 4 °C as free-floating sections before processing.

Post-mortem human brain tissue processing. A cohort of human brains (aged 10.3 mm below the skull surface. Animals received buprenorphine hydrochloride for postoperative pain control (Reckitt Benckiser).

Paraformaldehyde rat brain processing. Rats were anesthetized with ketamine and xylazine and perfused with 0.1 M PBS followed by 4% paraformaldehyde (PFA) in 0.1 M PBS. Brains were extracted, post fixed for 48 h in 4% PFA at 4°C, cryopreserved in 30% sucrose PBS solution and frozen in OCT compound on dry ice. Brains were stored at −80°C until cryosectioning.

Brain cryosectioning. Rat and human brain sections were acclimated to −20°C overnight and cut with a Leica VT1200S into coronally oriented 50-μm floating sections and stained following the same procedures used for rat brain processing. All specimens were co-stained with immunohistochemical staining with the support of the St. Joseph’s Hospital and Medical Center Committee on Human Research (Institutional Review Board approval no. 1081959; Reporting Summary).

Immunofluorescence labelling, confocal microscopy and MFI quantifications. Sections of rat and human brains (30 μm and 30 μm, respectively) were studied using antigen retrieval in 10 mM trisodium citrate (pH 8) and heated at 85 °C for 20 min. Immunostaining was performed according to published methods. Briefly, free-floating tissue sections were permeabilized for 25 min at room temperature (RT) in 0.1 M PBS ± 0.2% Triton X-100 and blocked for 2 h at 37°C in 0.1 M PBS ± 0.05% Triton X-100 (PBST) ±10% normal serum (Jackson Immunoresearch). Sections were then incubated overnight at 4°C using a 1:1,000 dilution of biotin-labelled WFA (L1516; Sigma-Aldrich), aggreCan (AB1031, Millipore), chondroitin-4-sulfate stub (MAB2030, Millipore), 1:250 of HAPLN-1 (AF2608, R&D), 1:50 of tenascin R (sc-376341, Santa Cruz Biotechnology) or 1:1,000 of parvalbumin (AP6598, R&D) antibodies in PBST + 1% donkey serum. For the following day, the sections were washed and incubated for 2 h at RT in 1:1,000 secondary antibodies in PBST + 1% serum. For studies investigating PNN changes in naïve and FGFI-treated rodent cohorts, parvalbumin was labelled with donkey anti-sheep 488 (A11015, Invitrogen), aggreCan was labelled with donkey anti-rabbit 555 (A31572, Invitrogen) and WFA was labelled with Streptavidin conjugate 647 (S32357, Invitrogen). Labelling was confirmed by repeating the staining using donkey anti-sheep 488 (A11015, Invitrogen), Streptavidin conjugate 555 (S32355, Invitrogen) and donkey anti-rabbit 647 (A31573, Invitrogen), or Streptavidin conjugate 488 (S32354, Invitrogen) and donkey anti-rabbit 394 (A21207, Invitrogen). For studies investigating ChABC targeting, aggreCan was labelled with donkey anti-rabbit 555 (A31572, Invitrogen) and WFA using Streptavidin conjugate 568 (S11226, Invitrogen). Sections were then counterstained with DAPI, mounted and cover-slipped using Fluoromount-G (4958-02, Thermo Fisher). Confocal microscopy was performed with Nikon A1R HS confocal objectives (×4, ×10, ×20), and fluorescence microscopy was performed with Zeiss Axio Observer 7 (ZEN 2.3 pro) objectives (×4). See the Report Summary for additional details.

To quantify PNN abundance, MFI of PNNs was assessed using an established method for PNN quantification and applied using a stereological approach. We first subdivided the Arc into three regions for analysis: (1) ArcM, (2) ArcL and (3) ArcV (ArcM+A, ArcL+B and ArcL+C). We conducted the study on the anatomical location of Arc neuronal populations of interest (for example, POMC and PVP)26,27. While the ArcM region is located 0.0–0.5 mm mediolateral to midline, the ArcL region is located 0.5–1.2 mm mediolateral to midline and lies lateral to the ArcM region and extends into both the ArcL and retrolenticular areas (which we have defined as ArcL for brevity). From each defined region of interest, we then subtracted a constant background and quantified the MFI (per mm²). Since PNN abundance varies throughout the rostrocaudal extent of the hypothalamus (Supplementary Fig. 1), we computed the averaged normalized intensity for PNNs by first normalizing PNN abundance to the controls for each region separately and then averaging the normalized changes for each stereotroical region. Data were blinded and images were processed for MFI using Fiji open-source software.

Hypothalamic CS/DS-GAG digestion and disaccharide isolation. CS/DS disaccharides were isolated and quantified according to our previously published methods. MBEl sections were isolated from 30-μm-thick PFA-fixed brain sections restricted to −1.6 to −2.4 mm from bregma. Fixed hypothalamic sections were washed 3x in Optima LC-MS-grade water and 1x in 50 mM ammonium bicarbonate (pH 7.6) at RT. ChABC digestion of CS/DS-GAGs was performed using 500 μl μl⁻¹ of ChABC (C3667, Sigma-Aldrich) in 50 mM ammonium bicarbonate (pH 7.6) in a Thermo Scientific MaxQ450 orbital shaker set to 80 r.p.m. at 37°C for 24 h. Supernatants were collected in sterile 1.7 ml microcentrifuge tubes and spun for 10 min at 14,000 g to pellet any debris. The supernatant was then dehydrated using a SpeedVac concentrator, and the lyophilized product was reconstituted in 30 μl LC-MS-grade water.

LC–MS/MS and MRM quantification of CS/DS disaccharides. CS/DS samples were analysed using a triple quadrupole mass spectrometer equipped with an electrospray ion source (Waters Xevo TQ-S) operated in negative mode and coupled to a Waters Acquity I-class ultra-high-pressure liquid chromatography system (UPLC). Disaccharides were resolved by porous graphic chromatography (Gradient 3 μm; Thermo Fisher) and collected in the ethane phase within 45 min. The MRM channels assigned were: ΔS-CS (CS-A) m/z 458 > 300, Δ4S6S-CS (CS-E) and Δ2S4S-DS (CS-B) m/z 538 > 300, ΔOS-CS (CS-O) and ΔHA m/z 378 > 175, Δ6S-CS (CS-C) m/z 458 > 282 and Δ2S6S-CS (CS-D) m/z 268 > 282. Masslynx software version 4.1 (Waters) was used to acquire and quantify all data. Under the conditions described above, the ratios between peak areas produced from equimolar CS standard run were normalized to the highest peak intensity, and relative quantification of each CS isomer within samples was achieved using a modified peak area normalization function. Each CS/DS isomer was expressed as a relative percentage of the total CS isomer composition within a brain sample.

Statistical analyses. For each study, treatment groups were matched for sex, age, body weight and blood glucose levels. Sample size calculations (Power3) yielded estimates of 4–10 per group predicated on ~80% power (alpha < 0.05, two-tailed), an end-of-treatment group glucose difference of 100 mg dl⁻¹, a pooled standard deviation of 55 and an allocation ratio of up to 2. Pairwise comparisons...
invoking immunofluorescence outcomes met the equality of variance assumption based on nonsignificant F-tests and were thus conducted using Student’s t-tests (unpaired, two-sided) in GraphPad Prism 7. Planned comparisons of outcomes measured at single time points were analysed using Student’s t-tests (unpaired, two-sided), and group by time outcomes (Extended Data Fig. 1) were analysed using linear mixed-effects analysis with Geisser–Greenhouse correction. Error bars represent the s.e.m. Investigators were not blinded to study conditions during the study, but were blinded during quantitative immunofluorescence analyses.

Lastly, we outline the analysis for the comparison of blood glucose levels over time in 1032 icv FGFl-treated ZDF rats receiving intrahypothalamic injection of either ChABC or heat-inactivated ChABC control. Because blood glucose declined to its lowest values between days 1–8 in both groups, and since this initial drop in glycaemia is known to result largely from the effect of icv FGFl injection to reduce food intake in ZDF rats39, the acute (days 1–8) and post-acute (days 10–28; no measurements on day 21) phases of glucose lowering were analysed separately. Group comparisons were made based on both the blood glucose AUC (computed using the trapezoidal rule41) and change of blood glucose levels over time. Group comparisons of body weight and food intake were likewise compared using the AUC, while baseline values were compared using the mean. Pairwise data were evaluated for equality of variance using the F-test, which was significant only for AUCG; thus, the Welch F-test was applied for that outcome because it is robust to unequal variance when the sample size differs42. To model the time-series data and assess group differences in blood glucose at discrete time points during the post-acute phase of the study, the data were analysed using a random intercept and slope linear mixed model43; the fixed effects were group, time, the group by time interaction and day of treatment. This model was adopted because blood glucose levels rose in a curvilinear fashion during the post-acute phase, and because of likelihood ratio goodness-of-fit tests comparing it to competing models44 and examination of regression diagnostics. Coefficient estimates and significance tests of group differences at discrete post-acute time points were computed by centring the interaction terms on the day in question45. AUC and regression analyses were performed using R46.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The data that support the findings of this study are available from the corresponding author upon request. Source data are provided with this paper.

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References

1. Mirzadeh, Z. et al. Perineuronal net formation during the critical period for neuronal maturation in the hypothalamic arcuate nucleus. Nat. Metabol. 1, 212–223 (2019).

2. Scarlett, J. M. et al. Central injection of fibroblast growth factor 1 induces sustained remission of diabetic hyperglycaemia in rodents. Nat. Med. 22, 800–806 (2016).

3. Scarlett, J. M. et al. Peripinonal net formation and ‘perineuronal nets’ in rat neocortex. Glu. 15, 131–140 (1995).

4. Schwartz, M. W., Woods, S. C., Porte, D. Jr., Seeley, R. J. & Baskin, D. G. Central nervous system control of food intake. Nature 404, 661–671 (2000).

5. Morton, G. J. & Schwartz, M. W. The NPY/AgRP neuron and energy homeostasis. Int. J. Obes. Relat. Metab. Disord. 25, S56–S62 (2001).

6. Morawski, M., Bruckner, G., Arendt, T. & Matthews, R. T. Aggrecan: beyond cartilage and skin. Cell. Biol. J. Br. Biochem. Cell. 68, 690–693 (1992).

7. Soares da Costa, D., Reis, R. L. & Pashkuleva, I. Sulfation of dermatan sulfate and growth factors/receptors/matrix proteins. Curr. Opin. Struct. Biol. 34, 36–47 (2015).

8. Li, Q. et al. Impaired cognitive function and altered hippocampal synaptic plasticity in mice lacking dermatan sulfotransferase Chst14/Dsst1. Front. Mol. Neurosci. 12, 26 (2019).

9. Alonge, A. M. et al. Quantitative analysis of chondroitin sulfate disaccharides from human and rodent fixed brain tissue by electrospray ionization-tandem mass spectrometry. Glycobiology 29, 847–860 (2019).

10. Miyata, S., Komatsu, Y., Yoshimura, Y., Taya, C. & Kitagawa, H. Persistent cortical plasticity by upregulation of chondroitin 6-sulfation. Nat. Neurosci. 15, 414–422 (2012).

11. Bouret, S. G., Draper, S. J. & Simler, R. B. Trophic action of leptin on hypothalamic neurons that regulate feeding. Science 304, 108–110 (2004).

12. Srinivasan, K., Viswanad, B., Atras, L., Kaul, C. L. & Ramarao, P. Inhibitory role of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. Pharmacol. Res. 52, 313–320 (2005).

13. Hamai, A. et al. Two distinct chondroitin sulfate ABC lysases. An endoelminase yielding tetrasaccharides and an exoelminase preferentially acting on oligosaccharides. J. Biol. Chem. 272, 9123–9130 (1997).

14. Miyata, S. & Kitagawa, H. Mechanisms for modulation of neural plasticity and axon regeneration by chondroitin sulphate. Biochim. Biophys. Acta. 15, 13–22 (2015).

15. Bruckner, G. et al. Acute and long-lasting changes in extracellular-matrix chondroitin-sulphate proteoglycans induced by injection of chondroitinase ABC in the adult rat brain. Exp. Brain Res. 121, 300–310 (1998).

16. Scarlett, J. M. et al. Perineuronal nets and chondroitin sulphate proteoglycans induced by injection of chondroitinase ABC in the adult rat brain. Exp. Brain Res. 121, 300–310 (1998).

17. Mauney, S. A. et al. Developmental pattern of perineuronal nets in the human prefrontal cortex and their deficit in schizophrenia. Biol. Psychiatry 74, 427–435 (2013).

18. Bhardwaj, R. K. & Yoo, T.-W. Perineuronal nets and schizophrenia: the importance of neuronal coatings. Neurosci. Biobehav. Rev. 45, 85–99 (2014).

19. Nadanaka, S., Clement, A., Masayama, K., Faisnser, A. & Sugahara, K. Characteristic hexasaccharide sequences in octasaccharides derived from shark cartilage chondroitin sulphate D with a neurite outgrowth promoting activity, J. Biol. Chem. 273, 329–3307 (1998).

20. Clement, A. M. et al. The DSD-1 carbohydrate epitope depends on sulfation. correlates with chondroitin sulphate D motifs, and is sufficient to promote neurite outgrowth. J. Biol. Chem. 273, 28444–28453 (1998).

21. Kruper, D. E. et al. Mechanosensing is critical for axon growth in the developing brain. Nat. Neurosci. 19, 1592–1598 (2016).

22. Xi, B. et al. Common polymorphism near the MC4R gene is associated with type 2 diabetes: data from a meta-analysis of 123,373 individuals. Diabetologia 55, 2660–2666 (2012).

23. Allcott, K. L. & Cone, R. D. The role of the central melanocortin system in the regulation of food intake and energy homeostasis: lessons from mouse models. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 361, 1265–1274 (2006).

24. Bhardwaj, R. K. & Yoo, T.-W. Perineuronal nets and schizophrenia: the importance of neuronal coatings. Neurosci. Biobehav. Rev. 45, 85–99 (2014).

25. Wang, X. et al. Variability in Zucker diabetic fatty rats: differences in disease progression in hyperglycemic and normoglycemic animals. Diabetes Metab. Syndr. Obes. 7, 531–541 (2014).

26. Slaker, M. L., Harkness, J. H. & Sorg, B. A. A standardized and automated method of perineuronal net analysis using Wisteria floribunda agglutinin staining intensity. IBRO Rep. 1, 54–60 (2016).

27. Elías, C. F. et al. Leptin directly regulates NPY and POMC neurons projecting to the lateral hypothalamic area. Neuron 23, 775–786 (1999).

28. Elías, C. F. et al. Chemical characterization of leptin-activated neurons in the rat brain. J. Comp. Neurol. 423, 261–281 (2000).

29. Schindelin, J. et al. Fiji: an open-source platform for biological-image analysis. Nature Methods 9, 676–682 (2012).

30. Osaghi, H. et al. Quantitative analysis of glycosaminoglycans, chondroitin/dermatan sulfate, hyaluronic acid, heparan sulfate and keratan sulfate by liquid chromatography-electrospray ionization-tandem mass spectrometry. Anal. Biochem. 467, 62–74 (2014).

31. Yu, X. et al. A rapid and precise method for quantification of fatty acids in human serum cholesteryl esters by liquid chromatography and tandem mass spectrometry. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 960, 222–229 (2014).

32. Mazimore, G. M., Laird, N. M. & Ware, J. H. Applied Longitudinal Analysis. (John Wiley & Sons, 2004).

33. Zar, J. Biostatistical Analysis 3rd edn, (Prentice Hall, 1996).
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Author contributions
K.M.A., Z.M., J.M.S., M.A.B., A.F.L., W.A.B., T.N.W., M.G., G.J.M. and M.W.S. contributed to experimental design, data interpretation and manuscript preparation, with input from all authors. In vivo studies were completed by K.M.A.; additional in vivo studies were conducted by J.M.S. and J.M.B. Immunofluorescence of human hypothalamic tissue was completed by Z.M. and E.C.; K.M.A., A.F.L. and C.K.C. performed the hypothalamic immunofluorescence stereological mapping, quantitative data analyses and protein biochemical analyses. Mass spectrometry analysis was performed and evaluated by K.M.A., M.G. and A.F.L. Statistical analysis was performed by K.J.K. and K.M.A.

Competing interests
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Extended Data Fig. 1 | Diabetic Wistar rats have reduced PNN CS-GAG structures in the arcuate nucleus. Wistar rats were rendered diabetic by 2 wk of high fat diet (HFD) followed by a single injection of low-dose streptozotocin (STZ) (30 mg/kg sc) or citrate control and, blood glucose and body weight were monitored for 24 d after treatment. Immunofluorescent detection of WFA (PNN CS-GAGs) and aggrecan (PNN CSPG) in coronal sections of rat hypothalamus (30 µm) from c, normoglycemic citrate Wistar controls and d, age-matched, hyperglycemic HFD/STZ Wistar rats. Scale bar: 200 µm. e, Quantification of the mean fluorescence intensity of PNNs averaged from medial hypothalamic sections (-2.2 to -2.8 mm posterior from bregma) for ArcM and ArcL areas from citrate and HFD/STZ Wistar rats and normalized to the citrate control mean fluorescence intensity averages. f, HFD/STZ Wistar rats exhibit no change in the relative percentages of CS disaccharides compared to age-matched, citrate controls. *P < 0.05, **P < 0.01, ****P < 0.0001, versus citrate controls; (a, b) Linear mixed-effects model analysis with Geisser-Greenhouse correction, and (e, f) Student’s t-test (unpaired, two-sided); (a) p<0.0001, (b) p=0.0364, (e) WFA, ArcL p=0.0061; WFA, ArcM p=0.0467. Arc, arcuate nucleus; ArcM, medial Arc; ArcL, lateral Arc; s.c., subcutaneous; 3V, 3rd ventricle.
Extended Data Fig. 2 | Effect of a single icv injection of FGF1 on Arc PNN assembly in Wistar rats. Immunofluorescence imaging of Arc PNN CS-GAGs in Wistar rats 24 h after icv treatment with FGF1 (3 µg) or vehicle, where the vehicle treated controls were pair-fed to the FGF1 group. Quantification of the mean fluorescence intensity of PNNs averaged from medial hypothalamic sections for total Arc (Arc-M+L) areas (n=5 rats/group; mean ± SEM). **P < 0.01, compared to icv vehicle controls; Student's t-test (unpaired, two-sided); (A) p=0.0046. Arc, arcuate nucleus; ME, median eminence; 3V, 3rd ventricle.
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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

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Data collection

- Inverted microscopes Nikon A1R HD and Zeiss Axio Observer 7 (ZEN 2.3 Pro)

Data analysis

- GraphPad Prism (7.0 statistical software), R Core Team (2016), Fiji image analysis software (Version 2.0.0-rc-69/1.52p), and MassLynx software version 4.1 (Waters)

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Sample size
Animal sample sizes accounting for treatment inter-subject variability were determined based on prior published FGF1 work (Scarlett, Nature Medicine, 2016; Scarlett, Diabetes, 2019; Brown, Diabetes, 2019) and for rodent and human PNN immunofluorescence staining and image intensity inter-subject variability (Mirzadeh, Nature Metabolism, 2019).

Data exclusions
No data exclusions.

Replication
A) Mass spectrometry analyses were run in duplicate, on two different sets of chondroitin sulfate disaccharide isolates from the same cohort, across 3 cohorts, and found to be reproducible. B) Immunofluorescence labeling of extracellular matrices in diabetic ZDF rats compared to non-diabetic, age-matched controls were repeated across 2 separate cohorts. Changes in ECM labeling between groups were consistent across both cohorts, and each cohort was stained 2x for ECM factors (e.g. WFA CS-GAGs, aggrecan CSPG) to confirm reproducibility in labeling. C) Human hypothalamic extracellular matrix immunofluorescence labeling was repeated across 5 patients and all patients reproducibly exhibited ECM structures within their hypothalamus.

Randomization
Rats were allocated to experimental groups receiving different treatments by matching age, body weight, food intake and blood glucose. There were no other experimental groups that received treatments other than rats.

Blinding
Since the investigators oversaw both the experimental treatments and metabolic measurements of rats during the study, these investigators were therefore not blinded to study conditions during the study. However, investigators were blinded to study conditions during quantitative immunofluorescence analyses.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☐   | Antibodies            |
| ☑   | Eukaryotic cell lines |
| ☐   | Palaeontology         |
| ☐   | Animals and other organisms |
| ☑   | Human research participants |
| ☐   | Clinical data         |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☐   | ChIP-seq              |
| ☑   | Flow cytometry        |
| ☑   | MRI-based neuroimaging |

Antibodies

Primary antibodies included: Wisteria floribunda agglutinin (WFA) (L1516; Sigma-Aldrich, St. Louis, MO), aggrecan (AB1031, Millipore, Burlington, MA), chondroitin-4-sulfate "stub" (MAB2030, Millipore, Burlington, MA), HAPLN-1 (AF2608, R&D, Minneapolis, MN), tenasin C (sc-376341, Santa Cruz Biotechnology, Dallas, TX), and parvalbumin (AF5058, R&D, Minneapolis, MN). Secondary antibodies against rabbit included: Alexa Fluor 488 donkey anti-rabbit IgG (H+L) (A21206, Invitrogen, Carlsbad, CA), Alexa Fluor 488 goat anti-rabbit IgG (H+L) (A11008, Invitrogen, Carlsbad, CA), Alexa Fluor 555 donkey anti-rabbit IgG (H+L) (A31572, Invitrogen, Carlsbad, CA), Alexa Fluor 594 donkey anti-rabbit IgG (H+L) (A21207, Invitrogen, Carlsbad, CA), and Alexa Fluor 647 donkey anti-rabbit IgG (H+L) (A31573, Invitrogen, Carlsbad, CA). Secondary antibodies against mouse included: Alexa Fluor 555 goat anti-mouse IgG (H+L) (A11105, Invitrogen, Carlsbad, CA). Secondary antibodies against goat included: Alexa Fluor 647 donkey anti-goat IgG (H+L) (A21447, Invitrogen, Carlsbad, CA). Secondary antibodies against sheep included: Alexa Fluor 488 donkey anti-sheep IgG (H+L) (A11015, Invitrogen, Carlsbad, CA).

Validation

Wisteria floribunda agglutinin (WFA): 9 published citations on manufacturer's webpage (https://www.sigmaaldrich.com/catalog/product/sigma/L1516?lang=en&region=US), 5 citations on Labome (https://www.labome.com/product/MilliporeSigma/L1516.html) and labeling was validated in lab by Chondroitinase digestion of CS-GAGs from fixed brain tissue both in vitro and in vivo; aggrecan: 140 published figures on BenchSci (https://app.benchsci.com/product/EMD%20Millipore/AB1031?figures), 18 citations on Labome (https://www.labome.com/product/EMD-Millipore/AB1031.html) and 32 references on manufacturer's webpage (https://www.emdmillipore.com/US/en/product/Anti-Aggrecan-Antibody,MM_NF-AB1031#documentation); chondroitin-4-sulfate "stub": 26 references on manufacturer's webpage (https://www.emdmillipore.com/US/en/product/Anti-Chondroitin-4-Sulfate-Antibody-clone-BE-123,MM_NF-MAB2030#documentation), 17 citations on Labome (https://www.labome.com/product/EMD-Millipore/MAB2030.html), 3 published figures on BenchSci (https://app.benchsci.com/product/EMD%20Millipore/MAB2030?figures), and labeling was validated in lab by...
Animals and other organisms

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Laboratory animals

For comparison between diabetic and non-diabetic rats: male Wistar rats (Hsd:WI, Envigo, Indianapolis, IN), male Zucker Diabetic Fatty (ZDF) rats (ZDF-Lepfa/Crl, 370 obese, Charles River, Wilmington, MA) and male Zucker Diabetic Lean (ZDL) rats (ZDF-Lep+/?, 371 lean, Charles River, Wilmington, MA) strains were 5 and 19 wks of age. For HFD/STZ-Wistar rat cohort of T2D, male Wistar rats were placed on HFD at 8 wks of age and treated with STZ at 10 wks of age. For icv FGF1 studies: male ZDF rats arrived at 8-10 weeks of age, underwent surgery and recovery, then treated with FGF1.

Wild animals

This study did not involve wild animals.

Field-collected samples

This study did not involve samples collected from the field.

Ethics oversight

All laboratory animal work was performed in accordance with NIH guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Washington (Seattle, Washington), and all experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All experiments reported are in compliance with the ARRIVE guidelines.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics

Brain specimens were collected from 5 patients (age/sex/PMI/diagnosis): (1) 23y/F/8h/cervical spinal cord glioma, (2) 56y/F/10h/temporal lobe glioblastoma, (3) 59y/M/13h/severe traumatic brain injury, (4) 63y/M/4h/occipital lobe glioblastoma, (5) 65y/F/18h/frontal lobe hemorrhagic stroke. The 23y/8h patient sample was used for the representative hypothalamic images, with all specimens showing similar labeling for perineuronal nets using UWA and aggrecan staining.

Recruitment

Human brain specimens were collected from deceased patients undergoing autopsy, with no inclusion/exclusion criteria except the requirement of being notified in time to collect the specimen with a post-mortem interval less than 24 hours to optimize tissue quality. All patients had neurological injury, which could bias results, but while the mechanisms of injury and the chronicity of disease varied between patients, all patients showed similar labeling for hypothalamic perineuronal nets.

Ethics oversight

All specimens were collected with informed consent and in ethical compliance with the St. Joseph’s Hospital and Medical Center Committee on Human Research (Institutional Review Board approval no. 108N159).

Note that full information on the approval of the study protocol must also be provided in the manuscript.