

Lumbar Intrathecal Infusion of an siRNA Formulation via Cannulation in Rats Demonstrated Comparable Tissue Distributions and Improved Animal Response as Compared to Non-Cannulated Free-Hand Method

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PURPOSE

To compare the drug exposure, tissue distribution and animal response after two intrathecal (IT) siRNA delivery techniques, via a pre-implanted cannula or via free-hand administration.

METHOD(S)

Two rats with IT-catheter implanted between T13 and L1 and extended to L3-L5 were used in the present study (Charles River Laboratories). An siRNA formulated in sterile artificial CSF (aCSF) was infused via a pump at approximately 1 µL/sec to conscious rats with a total volume of 30 µL. Pressure was held for an additional 1-2 minutes before the catheter was locked by sterile aCSF. A modified Mini-Irwin test was performed 24 hrs post dose and terminal collections including brain (hippocampus, prefrontal cortex, striatum, and cerebellum), spinal cord (cervical, thoracic, and lumbar regions), kidney, and liver were performed. Levels of the siRNA, as expressed as its antisense strand, in all tissue samples were quantified via a LC-MS/MS method based on a calibration curve prepared in rat brain homogenate (LLOQ = 11.15 ng/mL antisense strand (AS)). Historical data collected from five non-cannulated rats, intrathecally dosed with the same siRNA and sacrificed 14 days post-dose were used in the comparisons.

RESULT(S)

Both animals treated with the siRNA via cannulated IT administration showed detectable siRNA levels in all tissue types (central and peripheral) examined in this study. There is no observable difference noted between cannulated and non-cannulated rats in terms of tissue distribution and exposure of the siRNA. Rats that underwent cannulated IT administration exhibited normal, undisturbed behaviors 24 hrs post dose with normal auditory reflexes and tail suspension ability. No noticeable stress signals were observed during and after the IT infusion.

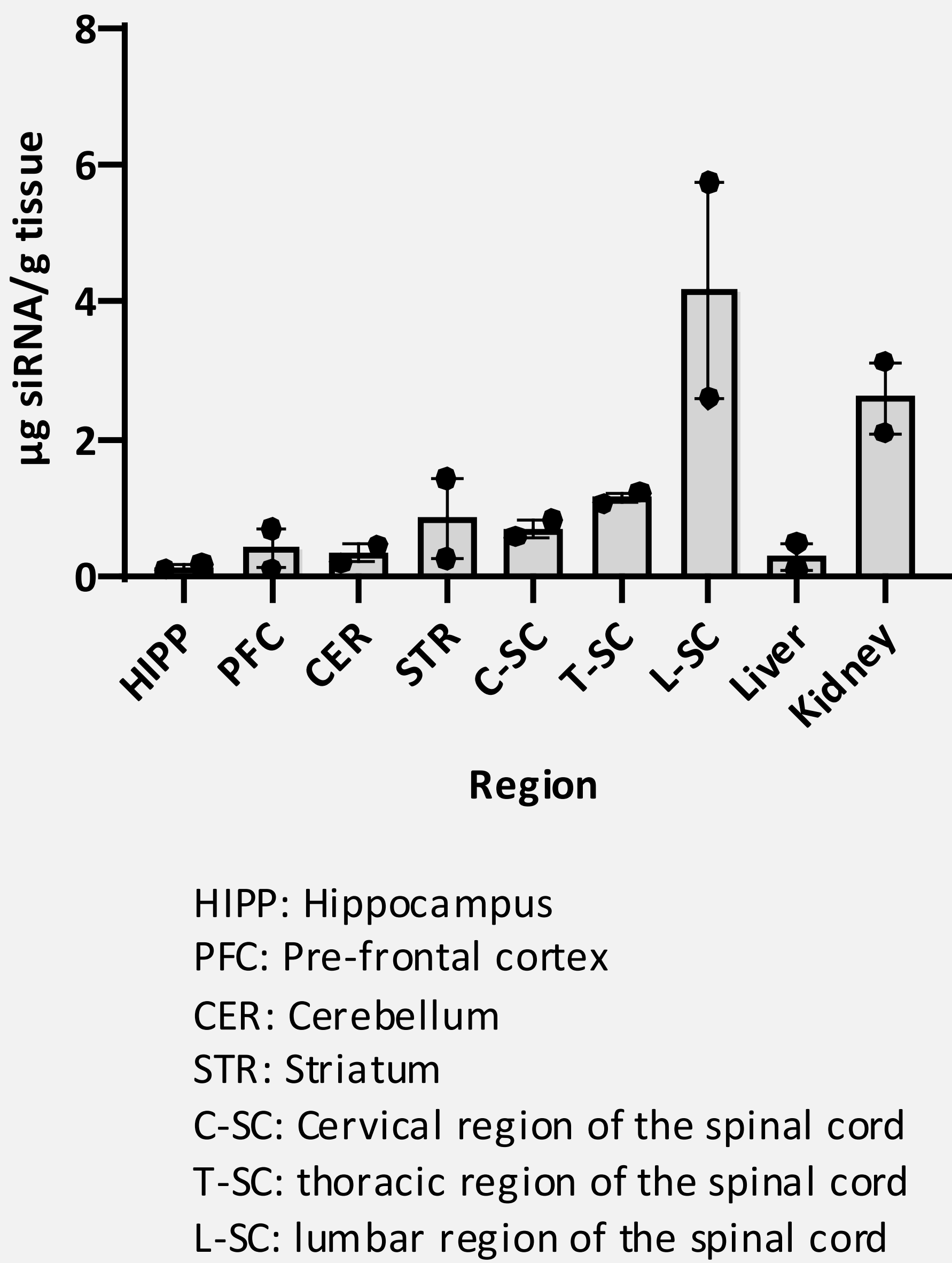


Figure 1 Tissue Exposure of the siRNA in Rat Brain, Spinal Cord, and Peripheral Tissues from the IT-Cannulated Administration

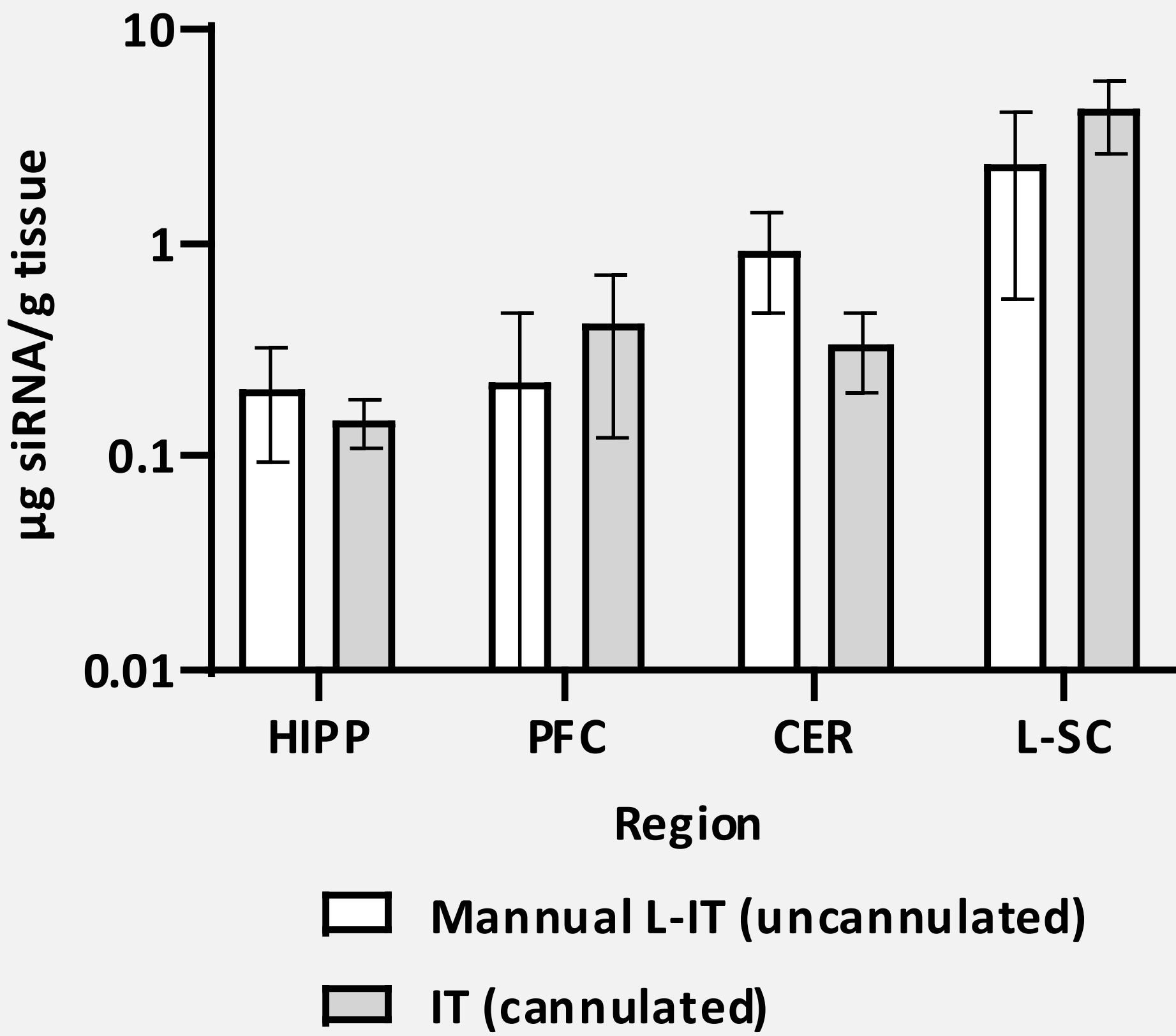


Figure 2 Tissue Exposure Comparison of the siRNA in Rat Brain and Spinal Cord from Cannulated vs. Non-cannulated IT-Administrations

Table 1 Post Administration Mini-Irwin Functional Observation Battery Test in Cannulated IT-dosed Rats

Endpoints/Animal #	1	2
Respiration Scores as normal, increase, decrease	Normal	Normal
Head twitches or flick Score as absent or present	Absent	Absent
Body position Score as normal, increase, decrease	Normal	Normal
Locomotor activity Score as normal, increase, decrease	Normal	Normal
Tremor Scores as absent or present	Absent	Absent
Sedation/excitation Score normal, sedation or excitation	Normal	Normal
Twitch Scores as absent or present	Absent	Absent
Seizures Scores as absent or present	Absent	Absent
Piloerection Scores as absent or present	Absent	Absent
Ptosis (eyelid closure) Scores as absent or present	Absent	Absent
Exophthalmos (protrusion of the eyeball(s) Scores as absent or present	Absent	Absent
Writhing (abdominal stretching) Scores as absent or present	Absent	Absent
Auditory Reflex Score as normal, increased or decreased	Normal	Normal
Tail Suspension Score as normal (slight struggle) increased (hang with twisting decreased (no struggle)	Normal	Normal

CONCLUSION(S)

The IT-cannulation delivery resulted in comparable siRNA exposures in rat brain and spinal cord. No noticeable stress was observed in the cannulated animals at the time of IT infusion. The administration did not need the use of anesthesia. This technique may improve the overall animal welfare of lumbar intrathecal administration and study quality/consistency.

STATEMENT

The work conducted was reviewed and approved by WuXi AppTec’s Institutional Animal Care and Use Committee (IACUC) according to the Guide for the Care and Use of Laboratory Animals.

