Supporting Information

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SI Materials and Methods

Materials. Saxitoxin (STX) stock solution was supplied to us by the U.S. Food and Drug Administration. Acetonitrile, ammonium sulfate, bupivacaine hydrochloride, chloroform, HPLC-grade dexamethasone, sodium chloride, methanol, and octyl β-D-glucopyranoside (OGP) were from Sigma; 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), 1,2-distearoyl-*sn*-glycero-3-phosphatidylglycerol, sodium salt (DSPG), and 1,2-dimyristoyl-*sn*-glycero-3-phosphglycerol, sodium salt- (DMPG) were purchased from Genzyme. Tert-butanol was purchased from Riedel-de Haën.

Liposome Characterization. To assess drug content, liposomes were first destroyed by adding them in a 1:2 ratio to 100 mM OGP. The resulting solution was then analyzed as described in the main article and below.

STX concentration determination was based on the method of Bates et al. (1), in which STX is oxidized to fluorescent products. Samples, standards, or blanks (0.3 mL) and 30% hydrogen peroxide (0.05 mL) were mixed vigorously with 5.0 mL 1.0M NaOH and 4.7 mL Milli-Q water. After 40 min at room temperature, the mixture received 0.7 mL concentrated acetic acid. Fluorescence was measured in a 1 cm cuvette in a PerkinElmer LS-50B, 330-nm excitation, 380-nm emission, excitation and emission slits 10 nm.

The concentrations of DSPC, DSPG, DMPC, and DMPG were determined colorimetrically by the Bartlett assay (2), which assessed the amount of phosphorus after hydrolysis of the phospholipids, with 1 mole of phosphorus equivalent to 1 mole of phospholipids. Samples, standards or blanks (0.2 mL) were mixed in 0.4 mL 10N H_2SO_4, and heated to 175 °C for 1 h. Subsequently, 0.03 mL of 30% hydrogen peroxide was added, and samples heated to 175 °C for 1 h; 2.3 mL of 22 mM ammonium molybdate, 2.3 mL of 0.44 mM H2SO4 and 0.2 mL of 0.1 mM 1-amino-2-naphthol-4-sulfonic acid (ANSA; Sigma) were added, and samples were boiled at 100 °C for 7 min. Absorbance was measured at 830 nm.

Cell Viability Assay. Cell viability was assessed after adding drug- or particle- containing media, by a colorimetric assay (MTT kit, Promega G4100) at selected time points. At each time point 150 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide was added, and then cells were incubated at 37 °C for 4 h, then 1 mL solubilization solution (detergent) was added. Absorbance was read at 570 nm with a SpectraMax 384 Plus fluorometer (Molecular Devices) after samples were incubated in the dark overnight. Cells were also monitored visually to confirm the results of the assay. Each plate had wells that contained media without cells or other additives whose absorbance was subtracted from the rest of the plate as background. All groups were then normalized to those wells.

Assessment of Nerve Blockade. Thermal nociception was assessed by a modified hotplate test (3, 4). In brief, hind paws were exposed in sequence (left then right) to a 56 °C hot plate (Model 39D Hot Plate Analgesia Meter; IITC). The time (latency) until paw withdrawal was measured by a stopwatch. (Thermal latency in the un-injected leg was a control for systemic effects of the injected agents.) If the animal did not remove its paw from the hot plate within 12 s, it was removed by the experimenter to avoid injury to the animal or the development of hyperalgesia. The experimenter was blinded as to what treatment specific rats were receiving.

The duration of thermal nociceptive block was calculated as the time required for thermal latency to return to a value of 7 s from a higher value; 7 s is the midpoint between a baseline thermal latency of ≈2 seconds in adult rats, and a maximal latency of 12 s.

Motor strength was assessed with a weight-bearing test. In brief, the animal was held over a digital balance such that it could bear weight with 1 hind paw at a time. The maximum weight that it could bear was recorded. The duration of motor blockade was defined as the time for weight bearing to return halfway to normal from maximal block. The halfway point for each rat was defined as [(highest weight borne by either leg) − (lowest weight borne by blocked leg)]/2 + (lowest weight borne by blocked leg).

1. Bates HA, Kostriken R, Rapoport H (1978) A chemical assay for saxitoxin. Improvements and modifications. *J Agr Food Chem* 26:252–254.
2. Bartlett GR (1959) Phosphorus assay in column chromatography. *J Biol Chem* 234:466–468.
3. Kohane DS, et al. (1998) A re-examination of tetrodotoxin for prolonged duration local anesthesia. *Anesthesiology* 89:119–131.
4. Soares S, et al. (2005) Neuronal and glial expression of the adhesion molecule TAG-1 is regulated after peripheral nerve lesion or central neurodegeneration of adult nervous system. *Eur J Neurosci* 21:1169–1180.

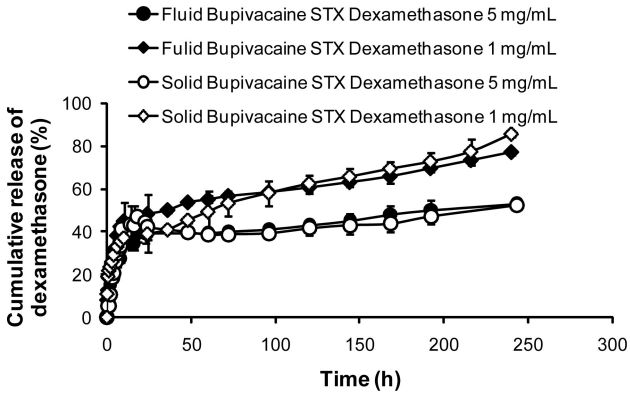

Fig. S1. Release of encapsulated compounds. Dexamethasone over time from liposome formulations in vitro expressed as a cumulative percentage of total encapsulated drug. Data are means with SDs ($n = 4$). Statistical significance is discussed in the text where relevant.

Table S1. Characterization of liposomes

Lipid composition	Compound concentration, mg/mL			Size, μm†	Zeta potential, mV
	Bupivacaine	Dexamethasone	STX		
DSPC, "solid"*	9.93 ± 0.54	—	—	4.0 ± 1.5	−32.2 ± 2.2
	10.22 ± 0.71	3.96 ± 0.11	—	4.1 ± 1.3	−30.2 ± 2.1
	—	—	0.031 ± 0.001	4.0 ± 1.2	−33.9 ± 2.1
	—	4.61 ± 0.23	0.027 ± 0.001	4.0 ± 1.6	−32.8 ± 2.2
	—	1.13 ± 0.24	0.029 ± 0.001	4.0 ± 1.4	−33.7 ± 2.1
	—	0.81 ± 0.03	0.030 ± 0.001	4.0 ± 1.2	−32.8 ± 3.2
	—	0.31 ± 0.01	0.030 ± 0.001	4.0 ± 1.3	−32.3 ± 2.4
	9.02 ± 0.2	—	0.022 ± 0.0005	3.8 ± 1.2	−36.0 ± 2.1
	9.91 ± 0.1	4.34 ± 0.06	0.021 ± 0.0004	3.9 ± 1.2	−35.6 ± 2.3
	8.86 ± 0.12	1.01 ± 0.035	0.022 ± 0.0001	4.0 ± 1.3	−34.6 ± 2.1
DMPC, "fluid"*	9.28 ± 1.1	—	—	4.2 ± 1.5	−33.6 ± 2.0
	8.74 ± 0.98	4.56 ± 0.76	—	3.9 ± 1.4	−33.6 ± 2.5
	—	—	0.030 ± 0.001	4.3 ± 1.0	−32.0 ± 2.3
	—	4.51 ± 0.97	0.026 ± 0.001	4.1 ± 1.8	−36.4 ± 2.4
	—	0.98 ± 0.02	0.028 ± 0.001	4.2 ± 1.3	−35.6 ± 2.0
	—	0.65 ± 0.01	0.029 ± 0.001	4.2 ± 1.1	−35.5 ± 2.1
	—	0.28 ± 0.01	0.030 ± 0.001	4.3 ± 1.0	−36.0 ± 2.0
	8.95 ± 0.87	—	0.019 ± 0.001	4.0 ± 1.1	−37.5 ± 1.2
	9.12 ± 1.21	4.60 ± 0.3	0.018 ± 0.002	3.8 ± 1.2	−33.0 ± 1.3
	8.84 ± 1.03	1.1 ± 0.04	0.019 ± 0.001	3.9 ± 1.0	−32.0 ± 1.1

Data are means ± SD, $n = 4$.

*The labels by which particles with these lipid compositions are referred to in the text.

†Median volume weighted diameter.