

Supplemental Methods

For some experiments, drug treatment was started at different times after MCAo. Drug infusions were delivered using a subcutaneously implanted miniosmotic pump (model 2001; 1.0 $\mu\text{L}/\text{h}$, Durect Corp, Cupertino, Calif), which was always implanted immediately after MCAo. To obtain the desired delay in start of treatment, pumps were fitted with a “Lynch coil”¹ consisting of a catheter containing air (“spacer substance”) that required the desired delay time to be replaced by the pump solution.

We performed control experiments to validate the performance of the “Lynch coils.” Miniosmotic pumps (model 2001; 1.0 $\mu\text{L}/\text{h}$, Durect Corp) were filled with either saline control (NS) or with 100 mg/mL phenol red in NS. The pumps were primed overnight at 37°C and then wiped with blotting paper and fitted with 4-hour delay “Lynch coils” (PE60, 7 mm beyond pump nipple). Pumps were placed into tubes containing 15 mL NS and incubated at 37°C. Every 30 minutes, a 200- μL aliquot was collected and placed in a well of a 96-well plate. Optical density was measured at 565 nM. This experiment was repeated in triplicate. A calibration curve to estimate released volume was constructed by adding 0.5 to 2 μL in 0.5- μL increments of phenol red solution (see previously) into separate tubes containing 15 mL of NS in triplicate. The data showing microliters of released volume from the pumps (mean \pm SE) as a function of time are shown (Figure).

Supplemental Reference

1. Lynch HJ, Rivest RW, Wurtman RJ. Artificial induction of melatonin rhythms by programmed microinfusion. *Neuroendocrinology*. 1980;31:106–111.

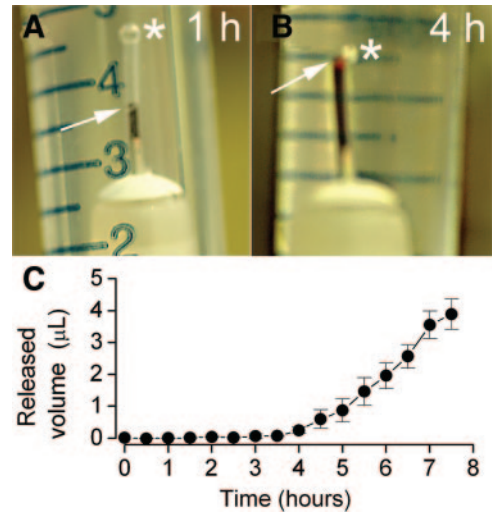


Figure 1. A–B, Photographs of “Lynch coils” attached to miniosmotic pumps obtained at approximately 1.5 hours (A) and approximately 4 hours (B) after start of the experiment; asterisks are next to small air bubbles being extruded from the tip of the “Lynch coils”; arrows point to the level of the phenol red solution in the “Lynch coils” at the indicated times. C, The volume of solution released from the pump as a function of time; n=3.

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