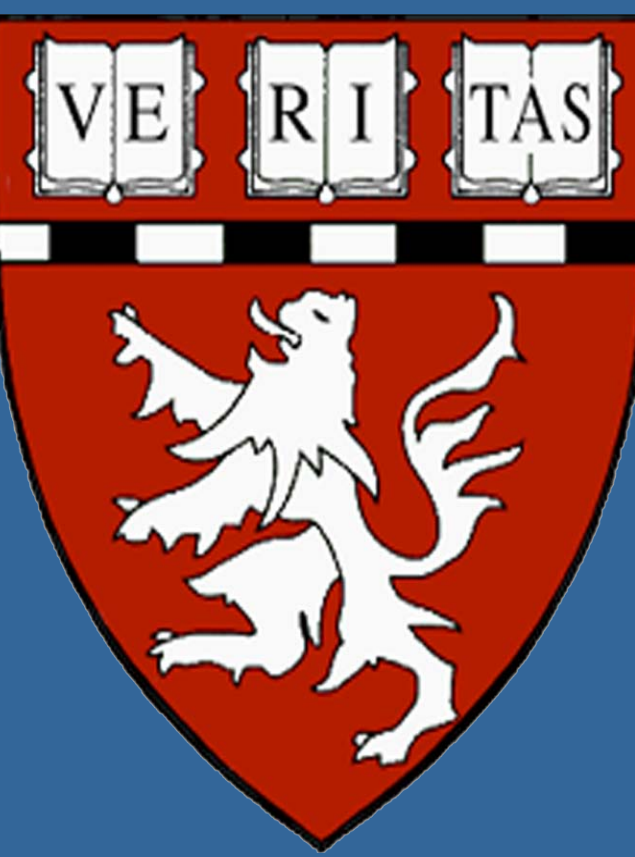


Vagus nerve stimulation in rat model of focal cerebral ischemia



I. AY*, H. AY, A.G. SORENSEN.

Athinoula A. Martinos Center for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA, 02129.

BACKGROUND

Parasympathetic system:
Extensively innervates cerebral vascular system and increases cerebral blood flow (CBF):
• Fibers originate from the superior salivatory nucleus in the pons,
• Run along the facial n. until they synapse in the sphenopalatine ganglion (SPG),
• Innervate blood vessels via the greater superficial petrosal n.

Other than CBF:
• Inhibits cytokine release ("cholinergic antiinflammatory pathway"),
• Reduces neuronal excitability.

Hypothesis: Modulation of brain's endogenous parasympathetic system may provide the opportunity to gain access to the region of ischemic but still viable tissue with critically impaired blood flow.

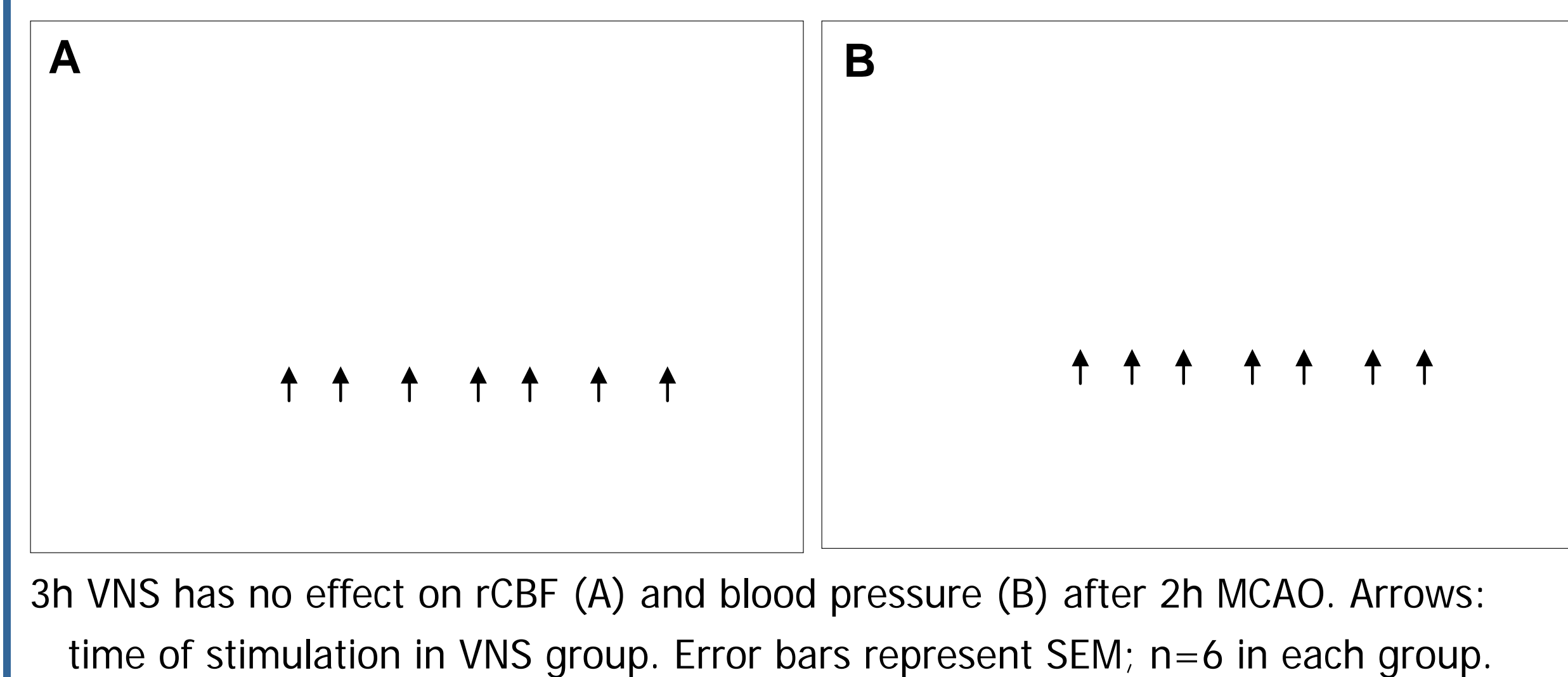
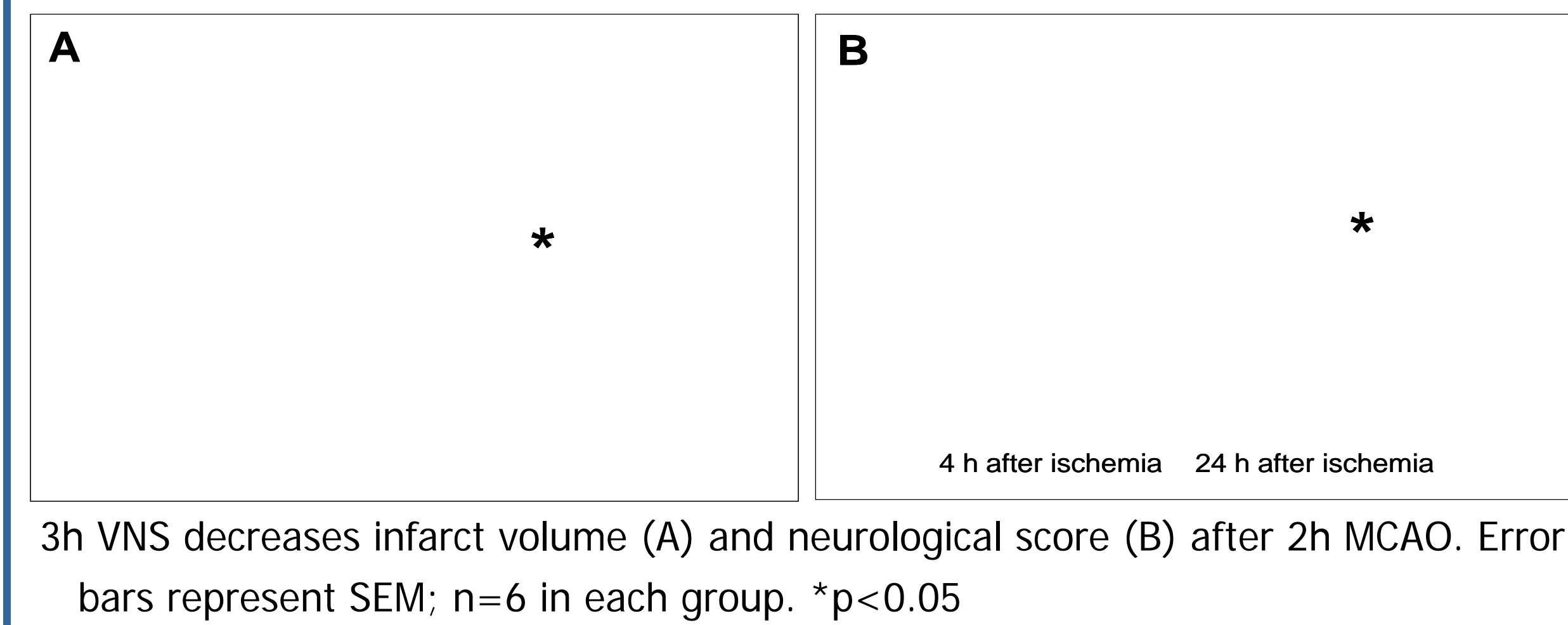
Unilateral SPG stimulation in rat focal ischemia reduces infarct size (Henninger and Fisher, 2007).

Vagus nerve stimulation (VNS):
• Safe and effective treatment for refractory partial onset seizures and treatment-resistant depression (George et al., 2002).
• Under study as a potential therapy for migraine, Alzheimer's disease, traumatic brain injury, and neuropathic pain (George et al., 2002; Smith et al., 2005).
• Decreases neuronal cell death in gerbil forebrain ischemia (Masada et al., 1996).

Aim: To investigate the effect of VNS after focal cerebral ischemia in rats.

Our Previous Studies:

3 h protocol
• Initiated 30 min after the induction of ischemia; repeated at every 30 min for 3 h
• 0.5 mA, 30 sec train of 0.5 msec pulses delivered at 20 Hz



Immediate and transient decrease in:
• MABP: 21.8 ± 1.3 mm Hg (n=42)
• Heart rate: 281.4 ± 17.8 beats/min (n=42)

The stimulation-induced reduction in MABP and heart rate lasted for only 30 seconds and completely returned back to normal at the end of the stimulation.

METHODS

Surgical procedures:

- Adult male Wistar rats (350-400 g, Charles River Laboratories)
- Isoflurane anesthesia
- Physiologic monitoring including rCBF
- Intraarterial filament occlusion of the right MCA for 2 h (Longa et al., 1989)

VNS:

- Initiated 30 min after the induction of ischemia
- Repeated at every 5 min for 1 h
- Stimulation parameters: 0.5 mA, 30 sec train of 0.5 msec pulses delivered at 20 Hz

Experimental groups:

- Treatment (n=6)
- Control (n=6): all procedures were duplicated but not stimulus was delivered

Neurological assessment:

- Five-point scale (0=no deficit – 4=no spontaneous walking) evaluation
- 4 h and 24 h after MCA occlusion

Determination of infarct size:

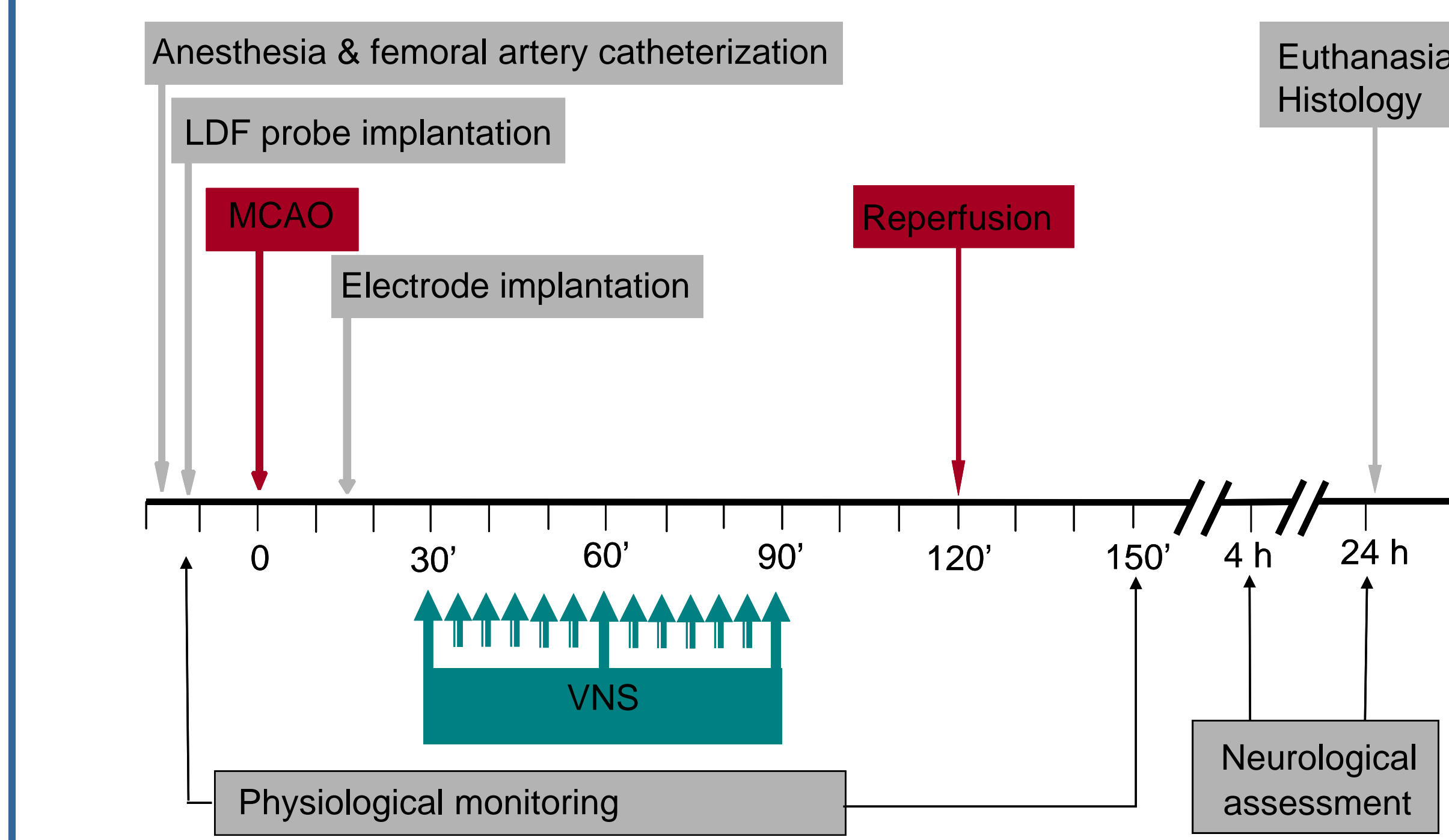
- 2 mm thick coronal brain sections; 2,3,5-triphenyltetrazolium chloride
- Infarct, ipsilateral non-infarct, and contralateral hemispheric areas were manually outlined by an investigator blinded to the treatment groups using Image J

- Infarct volume = infarct area (contralateral hemispheric area - ipsilateral non-infarct area) x slice thickness
- Infarct volume was expressed as a percentage of contralateral hemispheric volume

Data analysis:

- Data were expressed as mean ± S.E.M.
- Analyzed by repeated measures ANOVA or unpaired t-test
- P < 0.05 were considered statistically significant

Experimental Design



1 VNS has no effect on arterial blood gases and blood glucose before and after the MCA occlusion in rats.

			Before the MCA occlusion	60' after the MCA occlusion	After the reperfusion	150' after the MCA occlusion
ABGs	pH	Control	7.39 0.01	7.41 0.01	7.40 0.01	7.42 0.01
		VNS	7.40 0.01	7.40 0.01	7.40 0.01	7.42 0.01
	pCO ₂ (mmHg)	Control	44.2 2.13	42.1 4.86	41.0 2.43	37.4 2.15
		VNS	45.3 0.93	42.4 2.13	39.8 6.12	36.4 2.13
	pO ₂ (mmHg)	Control	87.2 3.96	88.9 3.72	95.7 3.15	102.4 3.21
		VNS	85.5 3.31	85.6 2.17	89.9 7.21	101.6 3.47
Blood glucose (mg/dL)	Control	122 5.47	121 4.36	120 3.48	118 3.95	
	VNS	121 8.13	121 4.12	119 2.43	117 5.65	

2 VNS causes an immediate and transient decrease in blood pressure and heart rate in rats.

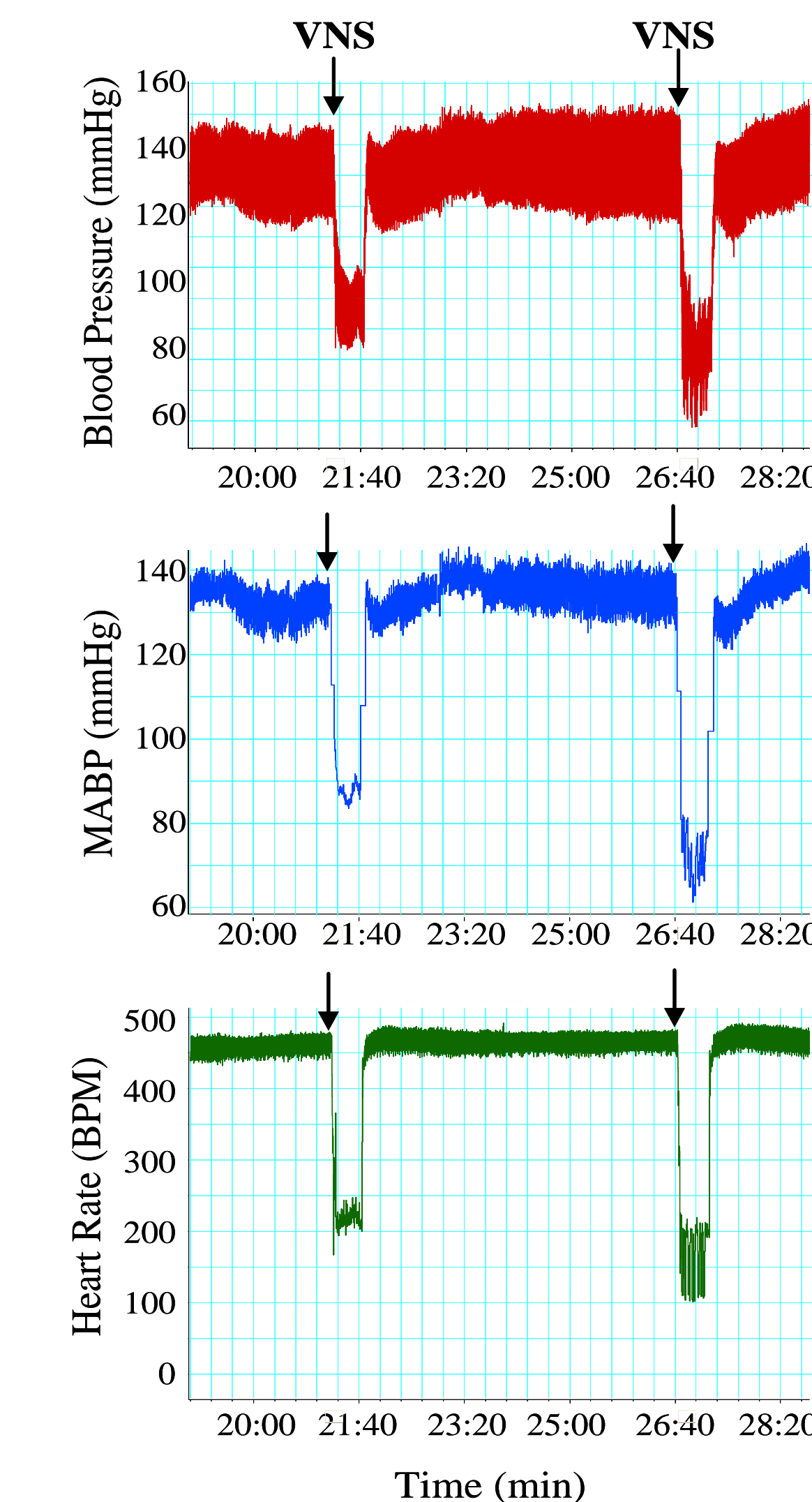
The decrease was observed in all the VNS-treated animals and with every stimulus.

The duration of this effect was around 30 seconds and completely reversible.

The amplitude of decrease in MABP was 47.8 ± 7.1 mm Hg (n=78).

The amplitude of decrease in heart rate was 272.3 ± 24.8 beats/min (n=78).

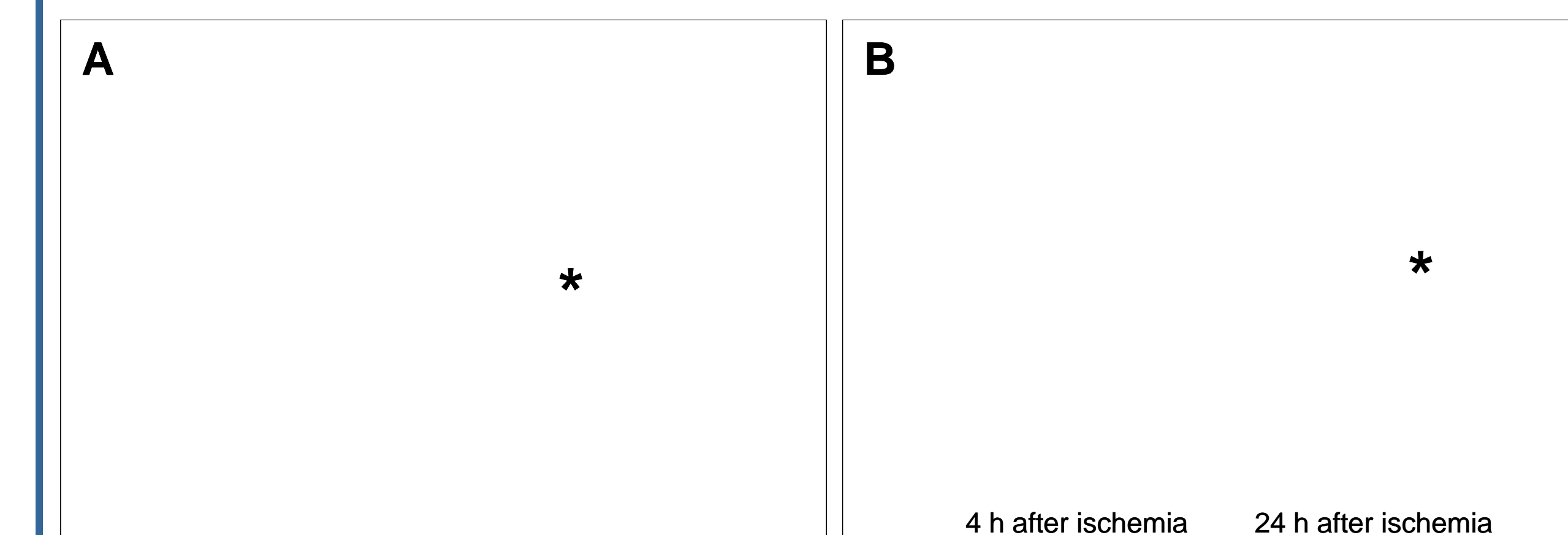
Representative recordings from a VNS-treated animal. Arrows show the time of electrical stimulation.



3 There was no difference between mean arterial blood pressure and heart rate of control and VNS-treated animals before and after the MCA occlusion in rats.

		MABP (mmHg)		HR (beats/min)	
Control	Before MCA occlusion	122.99	6.79	381.95	24.79
	150 min after MCA occlusion	113.31	6.02	418.39	30.74
VNS	Before MCA occlusion	104.33	4.13	366.27	12.17
	150 min after MCA occlusion	109.79	3.93	406.99	16.76
Repeated measures ANOVA		P=0.1073		P=0.6077	

4 VNS treatment decreases infarct size and improves neurological deficit in rat transient MCA occlusion.

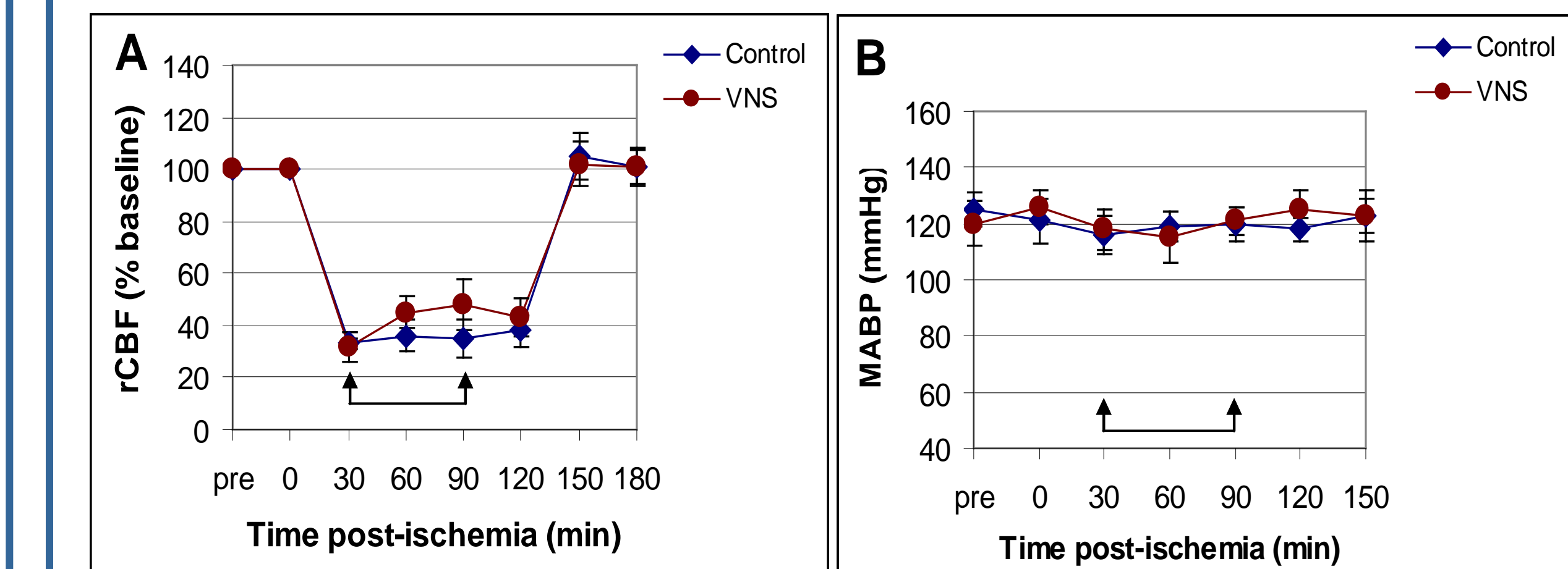


Effect of 1h VNS on infarct volume (A) and neurological score (B) after 2h MCAO. Error bars represent SEM; n=6 in each group. *p<0.05

- Infarct volume:
Control: 33.0 ± 5.0% of contralateral hemispheric volume
VNS: 16.2 ± 3.2% of contralateral hemispheric volume
Unpaired t-test: p=0.018

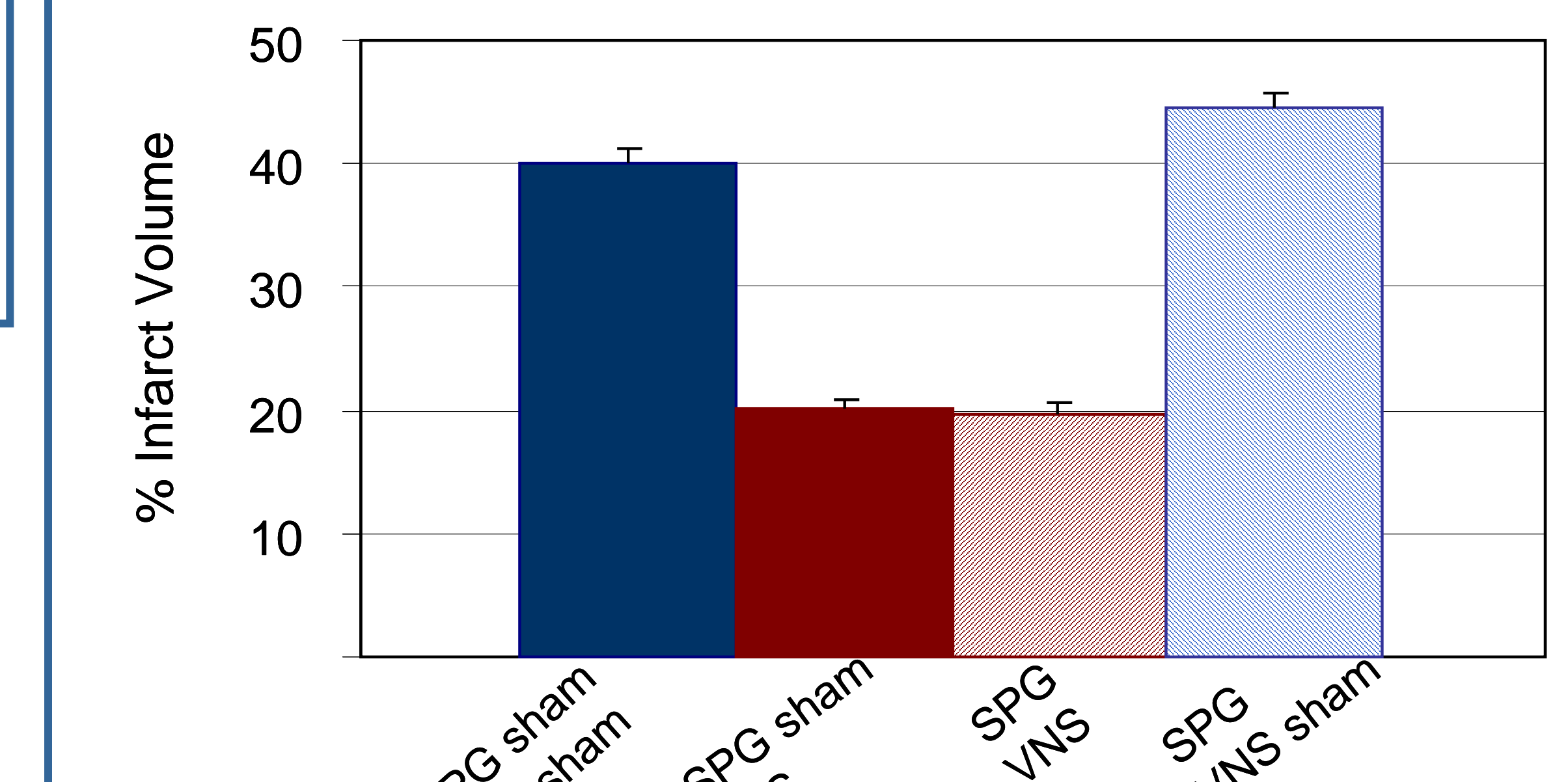
- Mean neurological score at 24h:
Control: 2.7 ± 0.2
VNS: 1.5 ± 0.2
Mann-Whitney U-test: p<0.05

5 VNS treatment does not change rCBF in rat transient MCA occlusion.



1h VNS has no effect on rCBF (A) and blood pressure (B) after 2h MCAO. Arrows: time period of stimulation in VNS group. Error bars represent SEM; n=6 in each group.

6 Infarct-reducing effect of VNS is not mediated through sphenopalatine ganglion in rat transient MCA occlusion.



1 week before the ischemia surgery bilateral sphenopalatine lesion (SPG) or sham surgery (SPG sham) was performed. MCAO reproduced as reported. In the treatment group 1h stimulation (VNS) was given. In the control group (VNS sham) electrodes were implanted but no stimulus was given. Error bars represent SEM; n=3 in each group.

CONCLUSIONS

In rat transient MCA occlusion, stimulation of right cervical vagus nerve:

1. has no effect on arterial blood gases and blood glucose,
2. causes transient decrease in arterial blood pressure and heart rate,
3. has no effect on cerebral blood flow,
4. decreases infarct size,
5. improves motor function of animals.
6. This effect is most likely not mediated by fibers from the sphenopalatine ganglion.

References:

1. Henninger N, Fisher M. (2007) Stimulating circle of willis nerve fibers preserves the diffusion-perfusion mismatch in experimental stroke. Stroke, 38:2779-2786.
2. George MS, Nahas Z, Bohning DE, Kozel FA, Anderson B, Chae JH, Lomarev M, Denslow S, Li X, Mu C. (2002) Vagus nerve stimulation therapy: A research update. Neurology, 59:556-61.
3. Smith DC, Modglin AA, Roosevelt RW, Neese SL, Jensen RA, Browning RA, Clough RW. (2005) Electrical stimulation of the vagus nerve enhances cognitive and motor recovery following moderate fluid percussion injury in the rat. J Neurotrauma, 22:1485-1502.
4. Masada T, Itano T, Fujisawa M, Miyamoto O, Tokuda M, Matsui H, Nagao S, Hatase O. (1996) Protective effect of vagus nerve stimulation on forebrain ischemia in gerbil hippocampus. Neuroreport, 7:446-448.
5. Longa EZ, Weinstein PR, Carlson S, Cummins R. (1989) Reversible middle cerebral artery occlusion without craniectomy in rats. Stroke, 20:84-91.

I.A. was supported by T32 Ruth L. Kirschstein National Research Service Award (5T32CA009502). A.G.S. was supported by PHS NS38477. Partial support was also provided by P41-RR14075 and the MIND Institute.
• A full listing of A.G.S.'s competing interests is available at www.biomarkers.org.