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# Increased BBB permeability by parasympathetic sphenopalatine ganglion stimulation in dogs

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**Abstract**

The blood–brain barrier (BBB) is a major obstacle for movement of large molecules to and from the brain. Stimulation of the sphenopalatine ganglion (SPG), the major source of parasympathetic innervation to brain vasculature, is known to vasodilate brain vessels, and has recently been shown to also increase the permeability of the BBB in the rat. In this work, we studied the effect of SPG stimulation on BBB permeability in larger animals—Beagle dogs. Left SPG was exposed by lateral approach in five Beagle dogs, and stimulated at 10 Hz. FITC labeled 10 kDa dextran was continuously infused to the left atrium during stimulation, and cerebral angiography was periodically obtained via the vertebral artery. Three control dogs received labeled dextran, without SPG exposure or stimulation. Brains were perfused with saline thoroughly at the end of stimulation, and samples from various regions were taken for fluorescence reading of tissue homogenates. Cerebral vasodilatation was evidenced in all but one dog, whose fluorescence results were consequently excluded from analysis, assuming that its SPG had been damaged by surgery. Fluorescence was significantly higher in the four stimulated compared to the three non-stimulated animals; e.g. mean FITC-dextran concentration in the anterior brain regions was $0.98 \pm 0.12$ ug (mean $\pm$ S.D.) FITC/g brain for experimental animals, and $0.40 \pm 0.02$ for controls ($p < 0.01$). No effect was seen in the pons and cerebellum ($0.68 \pm 0.22$ vs. $0.60 \pm 0.03$, NS) whose vascular innervation is supplied by the otic rather than the SPG ganglion. SPG stimulation appears to be an effective way to increase BBB permeability, allowing introduction of large molecules to the brain. This could be a therapeutic method for a wide variety of brain disorders, including tumors and neurodegenerative diseases.


*Keywords:* Blood–brain barrier; Parasympathetic; Sphenopalatine ganglion; Electrical stimulation

## 1. Introduction

The sphenopalatine ganglion (SPG), classically known to be the source of parasympathetic innervation to the nasal and eye mucosa and the lacrimal gland, is now recognized also as the source of parasympathetic innervation to the brain vasculature. It innervates the ipsilateral anterior cerebral circulation, and, in rodents, also some of the posterior and the contralateral circulation [2,6,7]. Electrical stimulation of SPG has been shown to induce vasodilatation of cerebral vessels in rat [7], cat [1], dog [11] and monkey [12]. This effect may involve secretion of NO [12]. An additional effect of such stimulation, described only recently by our group is a temporary increase in the permeability of the blood–brain barrier (BBB) [15]. The BBB is one of the major defense mechanisms of the brain, regulating blood–brain molecular traffic, and maintaining its delicate ionic and metabolic environment. Unless recognized by a specific transport system, hydrophilic molecules, and lipophilic molecules larger than ~500 Da, show extremely limited penetration across the BBB.

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The anatomy of the rat parasympathetic innervation of the cerebrum is quite different from that of humans. In the rat the parasympathetic fibers enter the cranial fossa via the ethmoidal foramen, accompanied by the nasociliary nerve, whereas in humans the spenopalatine ganglion, residing in the sphenopalatine fossa, is probably sending its fibers up via the cavernous sinus [5].

Aiming at human application of the SPG stimulation methodology, so far based on rat evidence only, the present study assessed the effects of SPG stimulation on the BBB in a larger animal. Dogs were used since their SPG, like humans, resides in the sphenopalatine fossa, and is supplied by the vidian nerve. Further, the animal is big enough so that stimulation can be applied selectively to the SPG.

## 2. Methods

Male Beagle dogs (body weight 10–14 kg) were used in this study, whose protocol was approved by the animal care and use committee at Shiga University of Medical Science. Dogs were anesthetized with intravenous injection of pentobarbital (30 mg/kg). Stable anesthetic conditions were attained by additional injections as needed. Transfemoral vertebral angiography was performed using a digital subtraction angiography system (DFA-3-30, Hitachi Medical, Tokyo, Japan) as previously described [10]. Transfemoral aortic catheter was used for delivery of the 10-kDa FITC-dextran conjugate tracer (p/n FD-10S, Sigma, Japan). Arterial blood pressure, heart rate and body temperature were continuously monitored.

The zygomatic arch and underlying muscles on the right side were excised, in order to reach the sphenopalatine fossa and make the SPG ganglion microscopically visible so that a fine, bipolar, concentric stimulating electrode could be inserted precisely into the ganglion. In order to verify the correct placement of the stimulation electrode, a 3-min stimulation period was given, during which we monitored cholinergic indications, ipsilateral lacrimation and nasal discharge. Then, a 2-ml aliquot of iopamidol was auto-injected (Angiomat 6000, Liebel-Flarsheim, OH, USA) into the right vertebral artery, whence five consecutive angiographic images of the circle of Willis were taken at 200 ms interval. Then, 15″ SPG stimulation was given (10 Hz, 6 V, pulse width 1 ms, monophasic, square wave). Seven seconds after stimulation commenced, the angiographic sequence was repeated. After 5 min, another angiographic sequence was performed without SPG stimulation. In each step, diameters of the anterior cerebral, middle cerebral, anterior communicating and posterior communicating arteries were recorded for later analysis of vasodilatation.

### 2.1. Delivery phase

The experimental group consisted of five dogs, three animals were in the control group, receiving FITC dextran without surgery or SPG stimulation. An additional animal was a fluorescence control, by not receiving FITC-dextran.

In the experimental group, 190 mg FITC-dextran of 10 kDa M.W. were continuously administered intra-aortically via a transfemoral catheter, using a programmed syringe pump, during the first 20 min following the beginning of SPG stimulation. Angiographic imaging was performed 5, 15 and 25 min following the start of SPG stimulation. Blood samples were collected at 10, 20, 30 and 40 min following the start of SPG stimulation, for fluorescence level.

Angiographic data obtained was stored in a digital data recorder (Hitachi Medical), and the diameter was measured by an image analyzer at selected two points (midpoint of the artery and 1 mm from the bifurcation with the circle of Willis), on both sides.

At the end of the experiment, the dogs' cephalic circulation was perfused using heparinized saline, through the aortic catheter, together with bilateral irrigation through both the common carotid arteries. Five minutes following the beginning of cephalic perfusion, the animal was sacrificed and the perfusion continued for an additional 15 min. Following the sacrifice the brain was taken out and biopsies were taken from the following regions: frontal cortex gray matter, frontal cortex white matter, cerebellar cortex, pons, olfactory bulb, striatum, hypothalamus, hippocampus as well as the optic nerve. Each part was homogenized in *heparinized saline*. The gingiva and the temporal muscle were also collected, as control tissues.

#### 2.1.1. Statistical analyses

For each animal, for each region, we computed the difference in FTIC readings between the left and right sides of the brain. Results are presented as means ± S.D., where S.D.s are computed across the all animals within each group (Experimental and Control). We then compared these differences between experimental and control animals by specifying the following multiple regression model, using PROC GLM (SAS®):

$$\text{Difference} = \text{Constant} + \text{Group} + \text{Region}.$$

There emerged significant main effects of Group and Region ($p < 0.01$ for each), which justified individual

Table 1
Increase (%) in diameter of middle cerebral artery (MCA) and posterior communicating artery (PCoA) during SPG stimulation

| Animal | MCA (% change) | PCoA (% change) |
|---|---|---|
| 1 | 32.3 | 19.2 |
| 2 | 16.9 | 6.2 |
| 3 | 23.7 | 10 |
| 4 | 15.2 | 7.8 |
| Mean ± S.D. | 20.3 ± 7.7% | 10.8 ± 5.8% |

Table 2
Fluorescent readings (ug FITC/g brain, mean ± S.D.) from brain homogenates of the left hemispheres

| Tissue | | Stimulated group ($N=4$) | Control group ($N=3$) | $P$ value | No FITC animal |
|---|---|---|---|---|---|
| SPG innervated | Frontal gray matter | 1.23 ± 0.08 | 0.44 ± 0.04 | <0.01 | 0.344 |
| | Frontal white matter | 0.95 ± 0.17 | 0.42 ± 0.04 | <0.01 | 0.335 |
| | Olfactory bulb | 0.93 ± 0.23 | 0.41 ± 0.05 | 0.01 | 0.320 |
| | Striatum | 0.81 ± 0.09 | 0.36 ± 0.03 | <0.01 | 0.268 |
| | Hypothalamus | 1.14 ± 0.43 | 0.40 ± 0.04 | 0.03 | 0.297 |
| | Hippocampus | 0.83 ± 0.19 | 0.38 ± 0.03 | 0.01 | 0.315 |
| | Optic nerve | 1.80 ± 1.21* | 0.75 ± 0.12 | | NT |
| Non-SPG innervated | Cerebellar cortex | 0.80 ± 0.41 | 0.62 ± 0.03 | 0.49 | 0.455 |
| | Pons | 0.57 ± 0.16 | 0.58 ± 0.04 | 0.98 | 0.466 |
| Non-BBB protected | Gingiva | 2.18 ± 0.61** | 2.77 ± 0.38 | >0.05 | NT |
| | Temporal muscle | 1.27 ± 0.38** | 1.69 ± 0.56 | >0.05 | NT |

*$n=2$, **$n=3$, NT = not tested.

comparisons between Experimental and Control animals for each region separately. These were done using independent $t$-tests. Alpha for significance was set at 0.05 throughout. Note that our model could not test the Group × Region interaction term for lack of degrees of freedom.

## 3. Results

Lacrimation and vasodilatation was observed in four of five experimental animals (Table 1). One experimental animal did not lacrimate, and showed minimal vasodilatation. We assumed this animal's SPG was damaged during its exposure, and excluded it from permeability analysis.

In the four experimental animals, stimulation of the SPG, significantly increased the fluorescent readings from the brain homogenates of the anterior parts of the brain, in the stimulated side, compared to controls (Table 2). No significant effect was shown for the pons and the cerebellum. Mean FITC-dextran concentration from anterior circulation tissues was 0.98 ± 0.12 ug FITC/g brain in experimental animals, and 0.40 ± 0.02 for controls ($p<0.01$), while for the posterior circulation tissues it was 0.68 ± 0.22 for experimental, and 0.60 ± 0.03 for controls ($p=0.52$). FITC-dextran concentrations in the anterior parts of the brain reached levels near those at the gingiva and temporal muscle, tissues not protected by the BBB. The unilaterality of the effect is demonstrated in Fig. 1, showing the side to side differences of FITC-dextran concentration in stimulated and control animals. Again it is noted that no difference is seen for the pons and cerebellum. The mean difference between sides for the SPG innervated anterior regions was 0.27 ± 0.04 ug FITC/

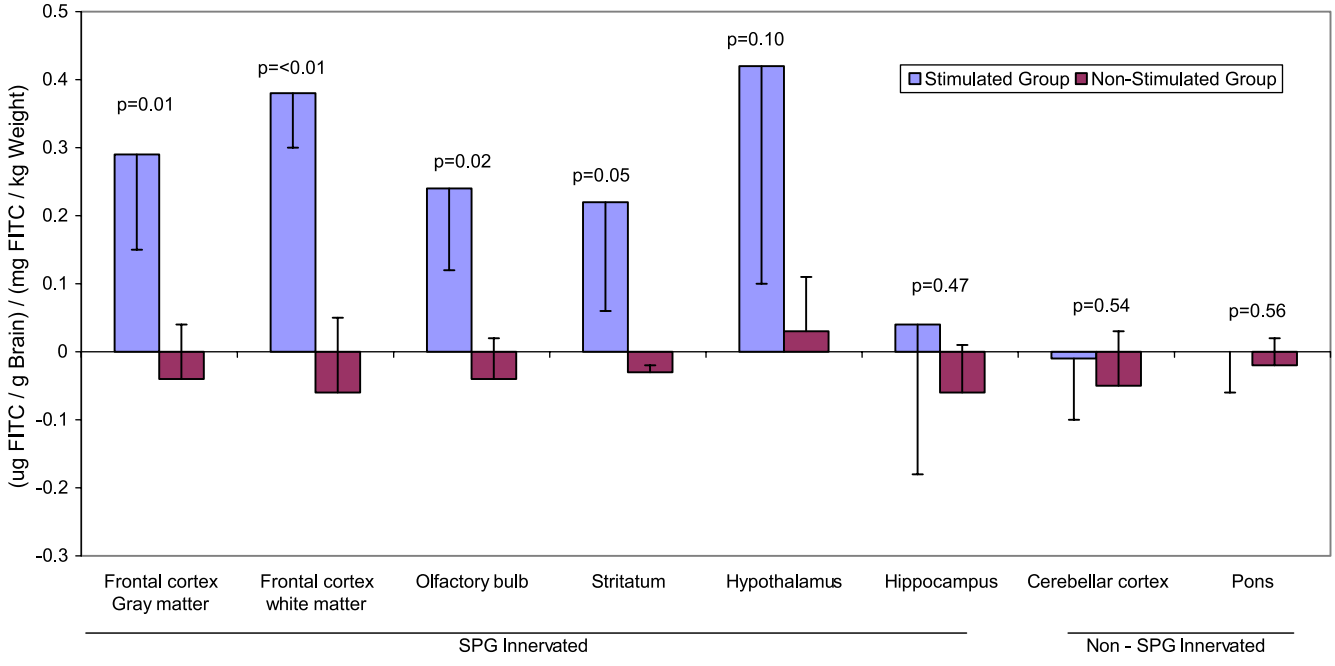


Fig. 1. Side to side differences for experimental vs. control animals: FITC concentrations in brain tissue ($N=4$, mean + S.D.).

g brain ($p < 0.01$), whereas a difference of $0.00 \pm 0.06$ was seen for the posterior regions ($p = 0.95$).

## 4. Discussion

SPG stimulation increases BBB permeability in the dog, markedly enhancing entry of the test macromolecule into the brain hemisphere on the stimulated side. This effect was apparent for the anterior parts of the hemisphere, those innervated by the sphenopalatine ganglion.

The sphenopalatine ganglion is mostly a parasympathetic one, and is the origin of innervation to the intracranial vessels, as has been recognized in the previous decade, when Suzuki et al. [7] and later Hara et al. [2] mapped the projections of parasympathetic fibers from the rat ganglion to most of the cerebro-vascular bed. A vasodilatory effect of SPG on cerebral vasculature was later shown by electrical stimulation of the ganglion [10,12]. Neurotransmission of this effect is probably through nitric oxide (NO), the neurotransmitter of the autonomic NANC (non-adrenergic non-cholinergic) functions [9], which is a potent vasodilator and inducer of plasma protein extravasation in many body systems. Mayhan [3] showed that administration of NO donors to brain vessels in the healthy animal causes increase in BBB permeability of these vessels (see also review by Thiel and Audus [8]). Further, Weyerbrock et al. [14] has shown such increase, induced by NO donors, also for C6 brain tumor model in the rat.

The present study confirms and widens the scope of our previous report on the effect of SPG stimulation on BBB permeability in rats [15]. We now show in dogs that permeability increases significantly with SPG stimulation. We measured FITC dextran concentration in several brain sites, and were able to show clear delineation between the anterior and posterior circulations, as only regions innervated by the SPG had a permeability increase, while the pons and cerebellum, whose vessels are innervated parasympathetically through the otic ganglion did not change their permeability. In humans, the hippocampus is a watershed structure, between the anterior and posterior choroidal arteries. A possible explanation for the lack of permeability increase in this study is that in the dog it is supplied mostly by the posterior circulation. This is unlike rats, where the parasympathetic fibers which enter the skull via the ethmoidal foramen appear to innervate all of the ipsilateral brain's vasculature, and to some extent the contralateral hemisphere as well. This unilateral effect in dogs, and also in the domestic pig (unpublished observations), suggest that in humans also, the effect of SPG stimulation would be unilateral.

An interesting finding is the increase in permeability of the optic nerve, considered an extension of the CNS based on its central myelin. Our results indicate that the BBB of this nerve can be overcome by SPG stimulation. This opens a potential therapeutic window for delivery of large molecules into this nerve.

Disruption of the BBB is of potential concern, due to the possible loss of the protective function of this barrier. This could lead to penetration of unwanted molecules, changes in the ionic, metabolic or osmotic balance, or invasion of immunogenic or infectious elements. Several safety tests have been carried and reported by our group [15] in rats. Water content of the brain did not increase significantly after SPG stimulation durations up to 24 h. The NAD/NADH balance of the brain did not change during and after stimulation, and TUNEL staining did not show any apoptotic cells subsequent to SPG stimulation. Beyond this experimental evidence, one can extrapolate from recurrent migraine attacks, which consist of neurogenic inflammation of the cerebral vessels, and probably cause a similar transient effect on BBB permeability [4], yet migraineurs do not show long-term brain damage. Similarly, most patients who contract meningitis or encephalitis sustain some transient damage to their BBB, usually lasting several days or more, and in most cases recover completely [13]. For these reasons, we conclude that application of this methodology to humans is feasible.

The extension of SPG stimulation to human could utilize the anatomy of the sphenopalatime fossa and its ramifications. The greater palatine canal is a bony canal that contains the greater palatine nerve and vessels, originating at the SPG, and reaching the oral cavity. The distal opening of this canal is in the hard palate, just medially to the third molar, and is easily approachable by a small mucosal incision. The potential applications are numerous, including the delivery of (i) chemotherapy for primary and secondary brain tumors, (ii) immune molecules, tailored to attack specific targets such as the amyloid protein in Alzheimer's disease, (iii) growth factors to stop degeneration and enhance regeneration in neurodegenerative diseases, such as Parkinson's, Alzheimer's, Huntington's, amyotrophic lateral sclerosis and others, and (iv) genes for modification of genetically based diseases. Further, the opening of the BBB could be utilized as a way to let out unwanted molecules, currently locked into the brain by the BBB in the brain. The beta-amyloid protein in Alzheimer's disease is a good example, as its washout into the blood could be predicted once the BBB permeability is increased. Further evaluation of the SPG stimulation effects, mostly from the safety point of view, is required, of course, before human application.

Cerebral vasodilatation by itself also has a potential clinical application. It can minimize the ischemic region in acute stroke, and prevent vasospasm in subarachnoid hemorrhage patients.

In summary, the present study shows that the BBB can be temporarily opened by electrical stimulation of the sphenopalatine ganglion in dogs. This is achieved concurrently with vasodilatation of brain vasculature. While human data is still not available, the extensive potential clinical applications, including brain tumors, neuro-degenerative and

immune-based brain diseases, call for future human application of this technique.

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