

# Episcleral Venous Pressure and IOP Responses to Central Electrical Stimulation in the Rat

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**PURPOSE.** Histological evidence suggests a role for the central nervous system in controlling episcleral venous pressure (EVP). Based on prior studies that identified candidate regions in the brain stem, the present study assessed the effect of electrical stimulation at the location of the superior salivatory nucleus (SSN) on EVP in rats.

**METHODS.** Male Sprague-Dawley rats ( $n = 11$ ) were anesthetized using pentobarbital sodium (50 mg/kg intraperitoneally initially, supplemented intravenously [IV] as needed) and paralyzed with gallamine triethiodide (1 mg/kg, IV). The animals were artificially ventilated and the femoral artery and vein were cannulated for blood pressure measurement and drug administration. Carotid blood flow was measured with an ultrasound flow probe and heart rate with a cardiometer. IOP was measured through a cannula in the vitreous compartment and EVP was measured through a micropipette in episcleral veins using the servonull technique. After a craniotomy was performed, a unipolar stainless steel electrode was inserted into the brainstem at the coordinates of the SSN using a stereotactic instrument. Stimulations were performed at 20Hz, 9  $\mu$ A, 1 ms pulse duration, and 200 pulses.

**RESULTS.** Stimulation at the SSN coordinates increased IOP from  $10.6 \pm 0.4$  to  $11.8 \pm 0.6$  mm Hg ( $P < 0.01$ ) and EVP from  $7.8 \pm 1.3$  to  $10.7 \pm 1.1$  mm Hg ( $P < 0.01$ ). Mean arterial pressure, carotid blood flow, and heart rate remained unaltered.

**CONCLUSIONS.** The present study indicates that the SSN may participate in regulating EVP.

Keywords: episcleral venous pressure, superior salivatory nucleus, stimulation, IOP

Steady-state IOP can be described by the Goldmann equation as the relationship between aqueous flow, uveoscleral outflow, outflow facility, and episcleral venous pressure (EVP).<sup>1,2</sup> EVP is the pressure that has to be overcome for fluid to leave the eye via the trabecular outflow pathway and, in humans under normal conditions, EVP accounts for roughly 60% of IOP. Despite its importance for IOP homeostasis, the physiology of the episcleral venous pressure is poorly understood. The current de facto standard for measuring EVP is the venomanometer described by Zeimer et al.<sup>3</sup> and numerous studies by different investigators have reported EVP values using this device, most of them confirming a value for EVP of approximately 9 mm Hg.<sup>4–9</sup> Venomanometry, however, is discontinuous and requires topical anesthesia, which may make it difficult to discern neural control of EVP. For example, using a different technique, a recent study by Zamora and Kiel<sup>10</sup> found a decrease of EVP in response to topical anesthesia, suggesting the existence of a neural tone affecting EVP. Although the elements of a neural circuit remain unknown, there is additional evidence for autonomic innervation of the anterior episcleral circulation from histological studies in primates.<sup>11</sup> Furthermore, the angioarchitecture of the episcleral circulation, with its numerous arteriovenous anastomoses and few capillaries, appears to be well suited for active regulation of episcleral blood flow and thereby EVP.<sup>12</sup>

Recently, a study by Samuels et al.<sup>13</sup> demonstrated the potential of hypothalamic centers to influence IOP, but did not further investigate the local (i.e., ocular) mechanisms involved.

The brainstem, however, appears to be the first relay station for nerves supplying the eye, and, thus, the nuclei there are likely to be more specialized than higher-order centers in the diencephalon.<sup>14</sup>

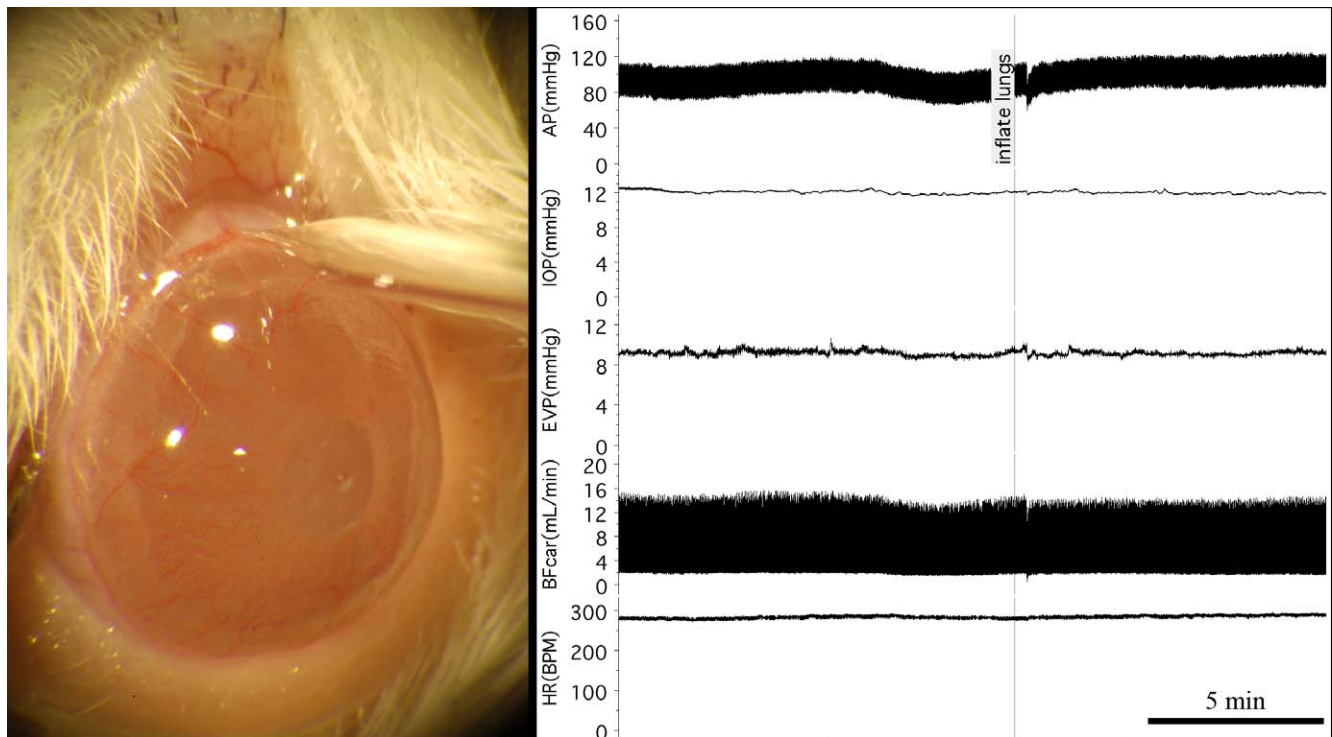
One candidate metencephalon nucleus is the superior salivatory nucleus (SSN), which has projections to the diencephalon and thence to the eye and choroid. In birds and rats, electrical stimulation of the SSN elicits choroidal vasodilation.<sup>15,16</sup> It is unknown whether the SSN projects to other ocular circulations. Therefore, the purpose of the present study was to determine the effect of electrical stimulation of the SSN on EVP and IOP.

## METHODS

The study was approved by the institutional animal care and use committee of the University of Texas Health Science Center at San Antonio and conducted in accordance with the ARVO guidelines for animal use in vision research. All animals were euthanized with an anesthetic overdose at the end of the experiment without regaining consciousness.

## Animal Preparation

Male Sprague Dawley rats ( $n = 11$ ,  $305.4 \pm 8.6$  g) were anesthetized using pentobarbital sodium (Sigma-Aldrich, 50 mg/kg intraperitoneally, supplemented intravenously as needed). A tracheotomy was performed and the animal was respired



**FIGURE 1.** Photograph of an episcleral vein cannulated with a servonull glass pipette and a 20-minute experimental trace indicating the relative stability of measured variables. Note that during an experiment lasting several hours, the lungs need to be inflated periodically to prevent atelectasis, which is indicated by the “inflate lungs” comment. Due to the increased intrathoracic pressure and impeded venous return to the heart, AP drops briefly.

with room air (SAR-830 small animal ventilator; CWE, Inc., Ardmore, PA). The preset weight-adjusted respirator settings were adapted to keep the end-tidal CO<sub>2</sub> between 35 and 45 mm Hg (CapStar 100 CO<sub>2</sub> Analyzer; CWE, Inc.). A catheter for blood pressure measurement (PE50 with a PE10 tip; Becton Dickinson, Sparks, MD) was inserted into the right femoral artery and connected to a pressure transducer (MLT 0380; AD Instruments, Colorado Springs, CO). The vitreous compartment was cannulated using a 27-gauge needle connected to a pressure transducer to measure IOP (MLT 0380; AD Instruments, Colorado Springs, CO). The presence of heart rate synchronous pulsations as well as respiratory changes in the IOP signal was considered as an indicator for successful cannulation. Another catheter (PE10) was inserted into the right femoral vein for drug administration. In some animals ( $n = 6$ ), a transit-time ultrasound flow probe was placed on the right common carotid artery to measure carotid blood flow (2PSB probe and TS420 flowmeter; Transonic Systems, Ithaca, NY). A digital cardiachometer triggered by the blood pressure or carotid blood flow signal was used to measure heart rate.

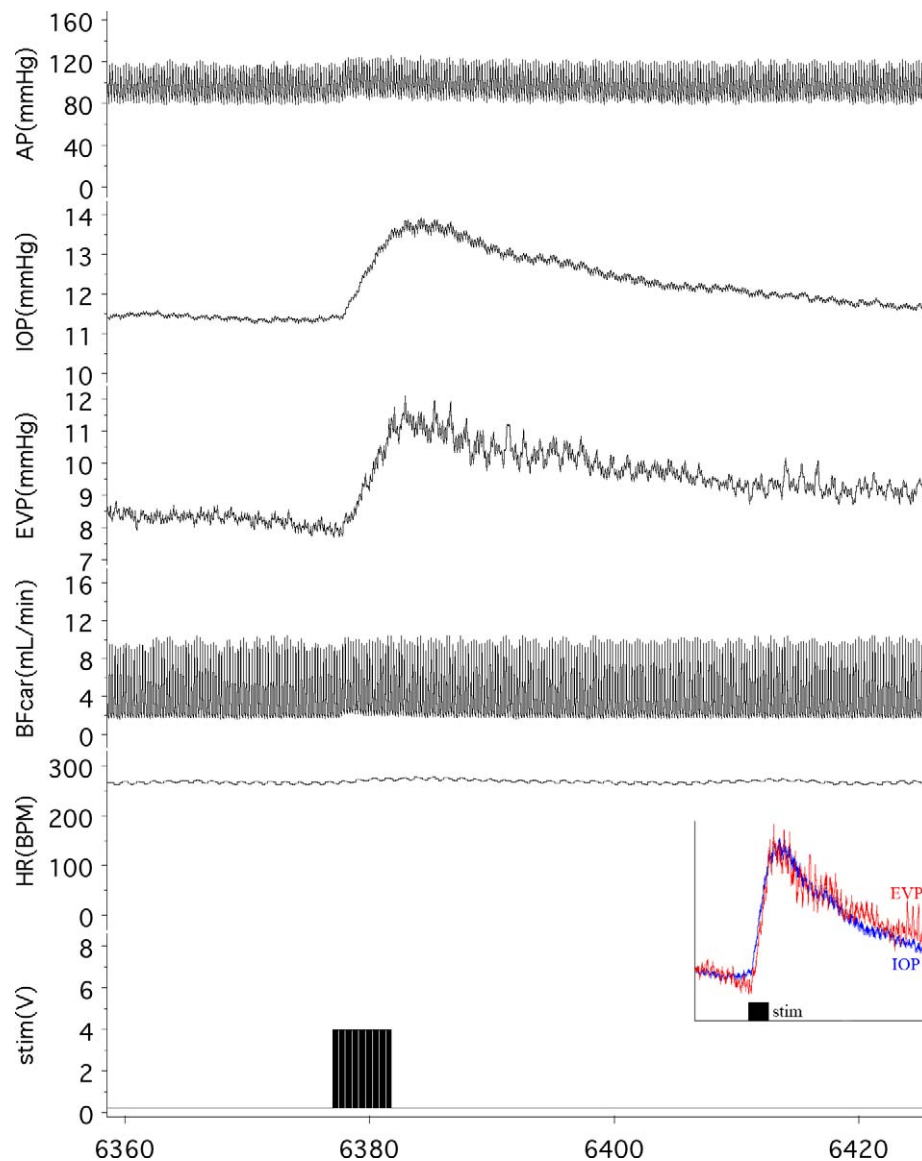
After the initial preparation, the animal was fixed in a stereotaxic head holder (Model 900; David Kopf Instruments, Tujunga, CA). Through a skin incision at the cranial midline, the skull was exposed and the Bregma point was calculated as described by Paxinos and Watson.<sup>17</sup> The SSN coordinates were calculated with regard to the Bregma position, 10.6 mm posterior, 2.2 mm lateral, and 9.5 mm below the bone surface. With a motorized tungsten-carbide drill (3.2 mm tip diameter), the bone was removed until the dura mater was visible. A layer of bonewax was applied to prevent bleeding once the dura mater was punctured. Then the stimulation electrode was advanced to the calculated coordinates.

To check the correct electrode position, a test stimulation was performed while a laser Doppler probe (PF403 connected to a PF500 flowmeter; Perimed, Stockholm, Sweden) was positioned on the anterior sclera, thus measuring an index of anterior choroidal blood flow. The height of the stimulation electrode was adjusted until a stable stimulation effect was achieved (i.e., anterior choroidal blood flow increased and discharge from the harderian gland was clearly visible).<sup>14,18</sup>

### EVP Measurement

EVP was measured with a micropipette-based servonull micropressure system (Model 900A; World Precision Instruments, Sarasota, FL). The servonull technique for EVP has been described in detail elsewhere<sup>19</sup>; hence, only a short overview is given here. Borosilicate glass pipettes (1B100-6; World Precision Instruments, Sarasota, FL) were drawn on a pipette puller and then beveled to sharpen the tip and achieve with a final tip diameter of 2 to 5  $\mu\text{m}$ . The pipettes were filled with a 2-M sodium chloride solution and connected to the servonull system. The ground wire was placed within a skin incision at the leg to close the electrical circuit.

The eyelid was retracted with two sutures at the lid margin to expose the episcleral vessels at the 12 o'clock position and a small incision in the conjunctiva was made above the target vessel to allow easy access for the glass pipette (see Fig. 1). A small drop of saline was then placed on top of the vessel and the pipette tip positioned in the saline to set the servonull system to zero. The saline was then removed and the pipette tip advanced into the episcleral vein. All procedures were performed under visual control using a surgical microscope.<sup>10</sup>



**FIGURE 2.** Representative tracing of AP, IOP, EVP, BFCar, and HR during electrical stimulation (stim) at the SSN coordinates. The systemic cardiovascular parameters remained unchanged, but IOP and EVP increased in response to the stimulation. The *color inset* shows the normalized EVP and IOP traces to illustrate the similarity of their temporal responses. (Time in seconds.)

### SSN Stimulation

After a stable baseline period for all measured variables, the SSN was stimulated electrically using a unipolar electrode (5710; A&M Systems, Carlsborg, WA) and a current controlled stimulator (FE180 Isolated Stimulator; AD Instruments). Stimulations were performed with a pulse train of 200 current pulses at an amplitude of 9  $\mu$ A, and a pulse duration of 1 ms with frequency of 20 Hz. This brief, 5-second stimulation period was chosen to minimize depletion of neurotransmitter reserves and possible damage to tissue at the electrode tip.

### Data Analysis

All parameters were recorded continuously on a digital recording system (PowerLab; AD Instruments) connected to a Mac Mini Computer (Apple, Cupertino, CA). The recording software package (LabChart 7, v7.2.5; AD Instruments) was used to determine the mean values of the measured variables during the minutes of stable baseline prior to stimulation and

then during and after the peak of the EVP response ( $\approx$ 5 seconds) to the stimulation.

Results are given as mean  $\pm$  SEM. Control and stimulation values for all parameters were compared using paired *t*-tests, with *P* less than 0.05 considered significant (Systat Software, Chicago, IL).

### RESULTS

Figure 1 shows a photograph of a typical experimental preparation with the superior lid retracted to expose an episcleral vein that was cannulated with a servonull pipette to measure EVP. The vitreous compartment was also cannulated temporally with a 27-gauge needle to measure IOP. Figure 1 also shows a 20-minute trace demonstrating the relative stability of the preparation and the measured variables. Figure 2 shows a representative tracing to illustrate the experimental protocol and the typical responses of the measured variables during electrical stimulation at the SSN coordinates in the brain

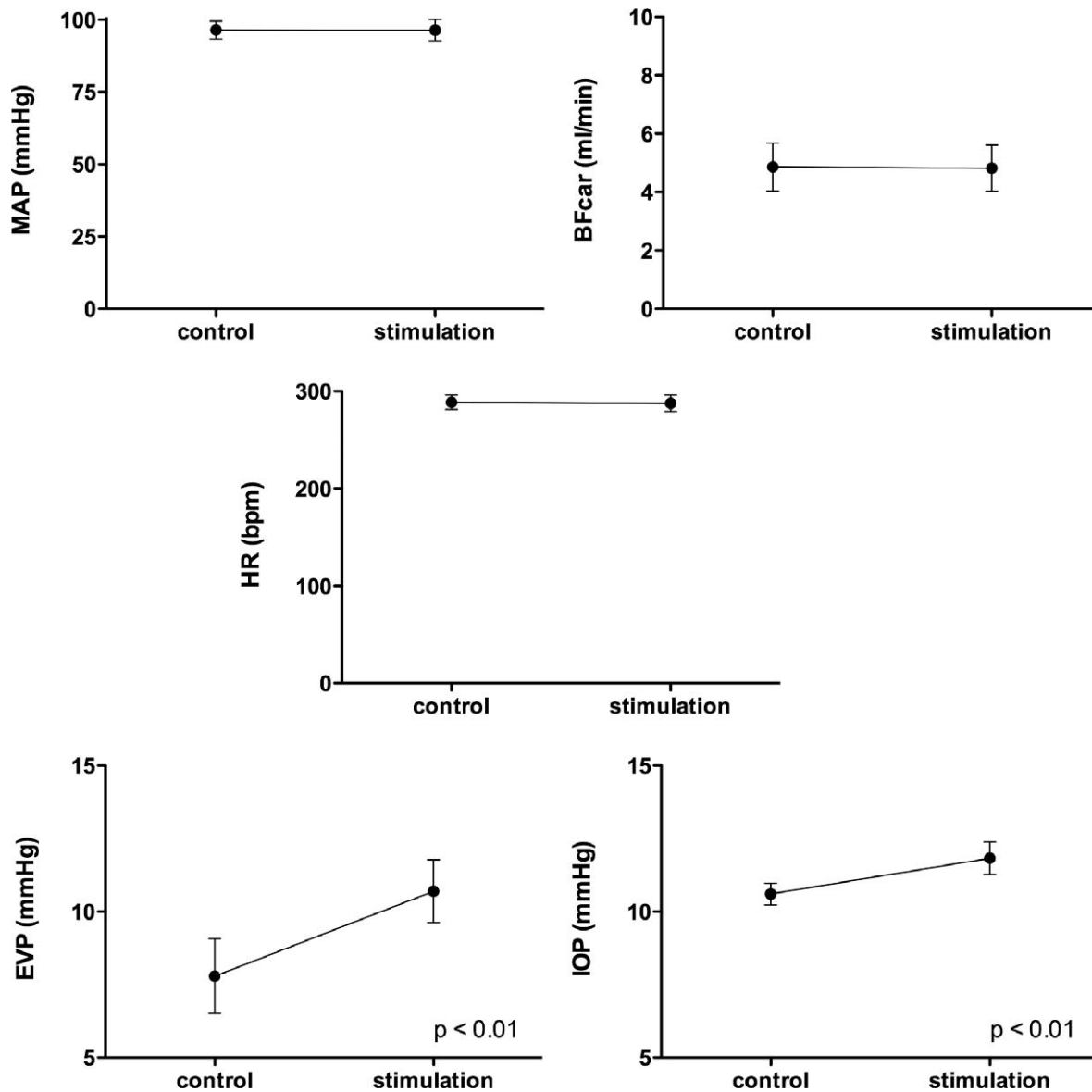


FIGURE 3. Responses of measured variables to stimulation at the SSN coordinates. EVP and IOP both increased significantly while systemic cardiovascular parameters were unchanged. All data are given as mean  $\pm$  SEM.

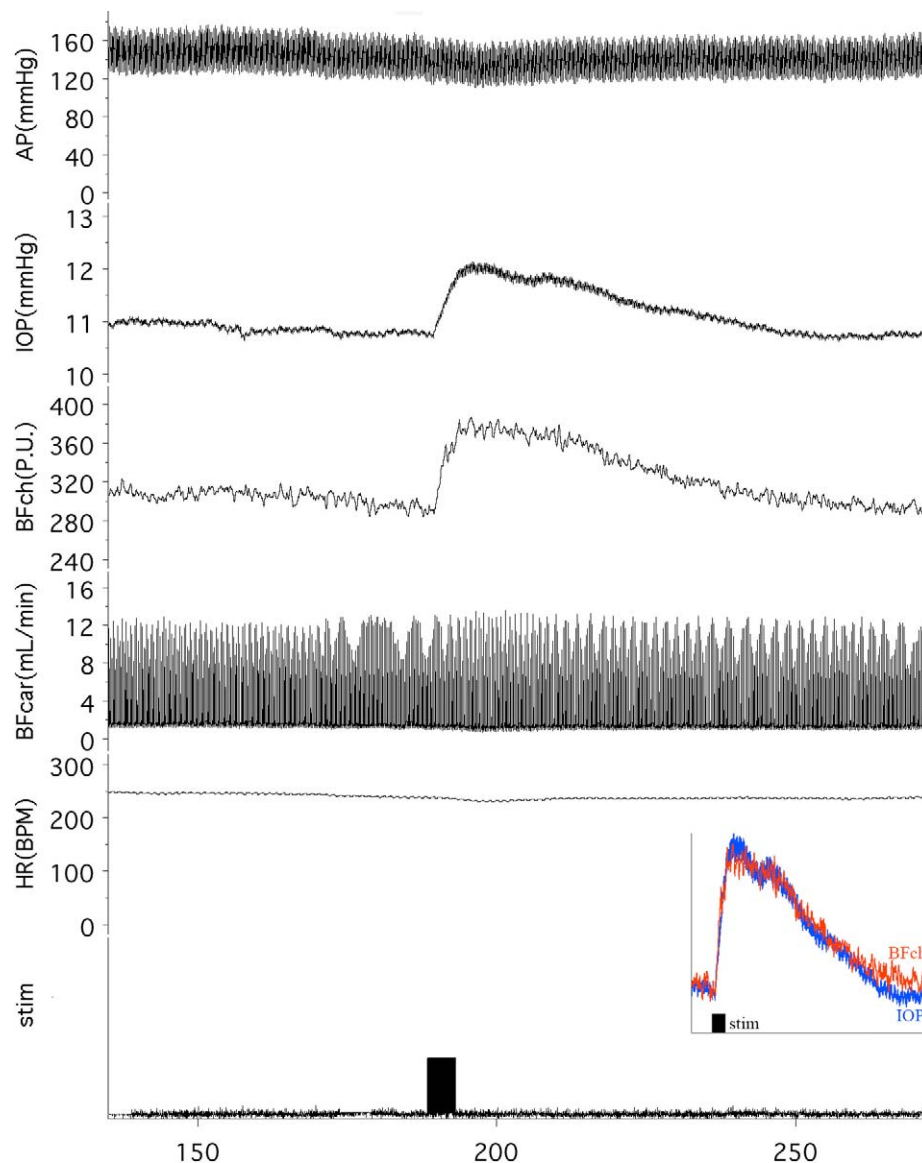
stem. The color inset shows an overlay of the normalized EVP and IOP traces indicating the similarity of their temporal responses.

Figure 3 graphically summarizes the responses of the measured variables to SSN stimulation. There was no change in mean arterial blood pressure (MAP;  $96.5 \pm 3.1$ - $96.4 \pm 3.3$  mm Hg,  $P = 0.97$ ,  $n = 11$ ), carotid blood flow (BFcar;  $4.9 \pm 0.8$ - $4.8 \pm 0.8$  mL/min,  $P = 0.732$ ,  $n = 6$ ), or heart rate (HR;  $289 \pm 7$ - $288 \pm 8$  beats per minute,  $P = 0.556$ ,  $n = 11$ ). By contrast, EVP increased by  $2.9 \pm 0.5$  mm Hg ( $7.8 \pm 1.3$ - $10.7 \pm 1.1$  mm Hg,  $P < 0.01$ ,  $n = 11$ ) and IOP increased by  $1.2 \pm 0.2$  mm Hg ( $10.6 \pm 0.4$ - $11.8 \pm 0.6$  mm Hg,  $P < 0.01$ ,  $n = 11$ ).

## DISCUSSION

The EVP is the pressure that has to be overcome for fluid to leave the eye via the conventional (trabecular) outflow

pathway and, therefore, it is an important determinant of steady-state IOP. Even though some species differences exist, the overall organizational principle of the outflow pathway, as well as the organization of the episcleral circulation, appears to be similar among primates, dogs, and rodents.<sup>11,12,20-22</sup> Furthermore, the anatomy of the episcleral vessels appears to be well suited for regulation of the pressure in the episcleral veins and thereby IOP, as the episcleral circulation is mostly devoid of capillaries and the arteries and veins are connected through anastomoses.<sup>11,12</sup> There is evidence that these anastomoses respond to changes in IOP in rabbits.<sup>23,24</sup> Even more interestingly, the anastomoses are supplied by nerves, whose nerve endings stain positively for autonomic neurotransmitters. A recent study by Zamora and Kiel<sup>10</sup> confirmed Ascher's<sup>25</sup> original observation that the episcleral circulation responds to topical anesthetics. Additional data supporting the assumption that the EVP is not passive, but actively regulated comes from a study investigating EVP changes during changes



**FIGURE 4.** Representative tracing of AP, IOP, choroidal blood flow (BFch), BFCar, and HR during electrical stimulation (stim) at the SSN coordinates. The systemic cardiovascular parameters remained relatively stable, but IOP and BFch increased in response to the stimulation. The *color inset* shows the normalized IOP and BFch traces to illustrate the similarity of their temporal responses. (Time in seconds.)

in body posture, where EVP changed less than predicted by the passive hydrostatic column effect.<sup>26</sup> The origin of episcleral innervation, as well as its function in the episcleral circulation are, to the best of the authors' knowledge, not addressed in the present literature.

There is, however, evidence for diencephalic centers influencing IOP,<sup>13,27–29</sup> but these studies did not measure EVP, and so the possible involvement of the episcleral circulation remains unknown. Moreover, the focus of investigation of those studies were diencephalic nuclei, which are commonly regarded as higher-order centers, and Li et al.<sup>14</sup> demonstrated that one population of vasodilatory nerves innervating the choroid in rats have their origin in the SSN in the brainstem, which appears to be the first-order relay station for neural control of choroidal blood flow.<sup>15</sup> Because it seemed plausible that an autonomic neural pathway to one ocular circulation might well go to others, in the present study we

sought to investigate the effect of electrical stimulation at the SSN location on EVP and IOP.

The main finding of the current investigation is that the episcleral circulation in rats responds to electrical stimulation at the coordinates of the SSN. EVP increased by  $2.9 \pm 0.5$  mm Hg in response to the stimulation, from a baseline value of  $7.8 \pm 1.3$  mm Hg. To our knowledge, there are no other data on EVP in rats, so it is unknown if the baseline EVP measured in this study is representative of the EVP in undisturbed rat eyes. However, the present findings are supported by similar values found in the past in small animals.<sup>30,31</sup>

More importantly, although the EVP response to brainstem stimulation suggests that EVP is under neural control, this result needs to be interpreted cautiously. The response does indicate that an efferent pathway capable of neural control of EVP exists, but it does not prove that EVP is actively regulated to achieve a particular set-point. Identifying a brainstem locus capable of altering EVP is an important first step in defining a

regulatory feedback loop, but future studies are needed to identify the afferent arm, the other sites of central integration, the regulatory set-point and gain, the efferent arm, and the end-organ effectors. Moreover, even if EVP is eventually shown to be neurally regulated, it may be difficult to prove that EVP regulation is an indirect means of controlling IOP. Nevertheless, it is tempting to speculate that IOP homeostasis may be its purpose and the possibility to influence such a regulatory loop, preferably locally through topical medication, provides sufficient impetus for further research.

Like EVP, IOP was also recorded and found to consistently increase in response to the stimulation at the SSN location, even though EVP rose more than IOP ( $P=0.008$ ). The fact that EVP and IOP increased simultaneously (Fig. 2 inset), as well as the difference in the effect size, argues against the IOP response being due to the rise in EVP and a simple explanation using the Goldmann equation. Although the rate of aqueous production and the rigidity coefficient in our rat preparation are unknown, an abrupt rise in EVP and consequent decrease in trabecular outflow takes some time (probably minutes) to increase ocular volume and increase IOP to a new steady state.<sup>32</sup> The brevity of the electrical stimulation (5 seconds) provided insufficient time for IOP to respond to EVP. The more likely explanation for the IOP response was an increase in choroidal blood flow<sup>14</sup> and concomitant choroidal engorgement. As noted in the Methods, we used the increase in choroidal blood flow to help verify electrode positioning prior to cannulating episcleral veins. Figure 4 shows the parallel increase in choroidal blood flow and IOP (inset), supporting the hypothesis that choroidal engorgement drove the IOP response. However, it is also possible that an unknown neurally regulated site downstream of the episcleral and choroidal circulations (i.e., vortex or orbital veins) was affected by the stimulation and contributed to the increase in EVP and IOP, although an increase in downstream venous resistance or pressure is inconsistent with the increased BFch.

The brevity of the stimulation is a limitation of this study, as steady-state conditions could not be evaluated. The selective investigation of central nuclei inevitably requires invasive surgery and crude instruments and devices in comparison with the structures studied. Additionally, electrical stimulation comes with a current spread, whose magnitude depends on the stimulation amplitude. Given the tightly packed arrangement of nuclei in the brain stem, with blood pressure controlling centers nearby (e.g., the A5 neuron group), we chose the smallest and shortest stimulation amplitude that yielded stable EVP effects. Nevertheless, in some animals, slight changes in blood pressure were seen. Animals with a change in arterial blood pressure greater than 10 mm Hg were regarded as having a misplaced stimulation electrode and were discarded from the study. Overall, there was no significant effect of the stimulation on blood pressure, carotid blood flow, or heart rate. Another related limitation is that we did not have the equipment to lesion and later histologically verify the electrode tip positioning in the SSN; however, the SSN coordinates according to Paxinos and Watson<sup>17</sup> were easily accessed and stimulation at that location elicited the anterior choroidal hyperemia and harderian gland discharge reported by others.<sup>14,18</sup>

In conclusion, the present study provides evidence that the SSN is part of a neural circuit capable of controlling EVP in rats. Studies to identify the other elements of this possible EVP-regulating feedback loop and its possible role in controlling IOP are warranted.

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