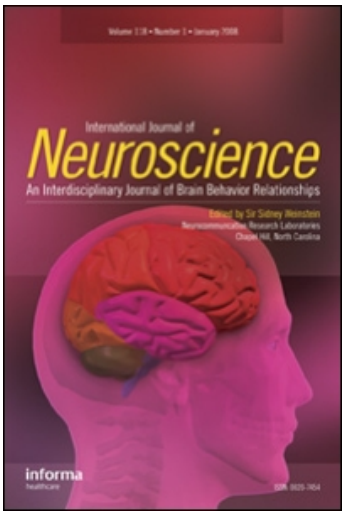

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The existence of centrifugal fibers projecting into the mammalian retina is well known. However, their precise physiological role is poorly understood. Here we report that stimulation of the dorsal raphe nucleus (DRN) in freely moving rats produces profound effects on the electroretinogram (ERG). Most notably, activation of the dorsal raphe–retinal pathway causes a significant decrease in the latency of the b-wave and accompanying oscillatory potentials. In addition, dorsal raphe stimulation leads to a significant increase in the amplitude of oscillatory potentials. These results, therefore, provide the first demonstration of a functional role for the retinopetal fiber system originating in the and suggest that this structure can exert a powerful influence over the temporal sharpness and efficacy of retinal responsiveness.

Keywords B-wave, electroretinogram, freely moving rats, oscillatory potentials, retinal efferents

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INTRODUCTION

The presence of centrifugal fibers projecting to the mammalian retina has been recognized for nearly a century (Cajal, 1911). These fibers, which are commonly referred to as retinal efferents, originate from different brain areas (Reperant, Ward, Miceli, Rio, Medina, & Kenigfest, 2007) and are of considerable theoretical and practical interest because they provide a means whereby the central nervous system might influence retinal ganglion cell output. However, despite possessing such an obvious potential to influence early stage visual processing, the precise physiological capabilities of these retinopetal fibers are not well understood.

In the current study, we focus on the specific retinopetal projection from the DRN (Fite, Fite, Bengston, & Cosentino, 1996; Labandeira-Garcia, 1988; Lima & Urbina, 1998; Villar, Vitale, & Parisi, 1987). The DRN is worthy of particular attention because the firing rate of neurons in this structure varies considerably according to the behavioral state. Specifically, during wakefulness these cells fire at a substantially higher rate than during slow wave and REM sleep, when firing is greatly diminished (Sakai & Crochet, 2001). Thus it appears that the DRN might be able to directly influence retinal processing in a behavioral state dependent manner. This is interesting because we recently reported that the amplitudes of the light-evoked ganglion cell responses of a freely moving rat vary in a systematic manner between wakefulness, slow wave sleep, and REM sleep (Galambos, Szabo-Salfay, Szatmari, Szilagyi, & Juhasz, 2001). Furthermore, we hypothesized that the retinopetal fibers originating from the DRN might be responsible for this variation.

In order to test whether activity in the DRN is able to directly influence retinal processing we recorded light-evoked responses from the retina and examined how these were modified by electrical stimulation of the DRN. It was found that for specific intervals between DRN stimulation and elicitation of light-evoked potentials there is a significant reduction in the latency of the b-wave and the associated oscillatory potentials (OPs) of the ERG. The OPs were also subject to a pronounced increase in amplitude. These results not only confirm that retinal functioning is under the direct control of the DRN but further suggest that the DRN may act specifically to increase its sharpness and efficiency.

METHODS

Experiments were performed in accordance with the guidelines of the European Communities Council Directive and the local ethics committee. Experimental

procedures are described in detail elsewhere (Szabo-Salfay et al., 2001). Briefly, six adult (250–350 g) male Sprague Dawley rats were implanted under halothane anesthesia with two electrically sharpened 80-μm bipolar tungsten electrodes (tip distance 0.5 mm; electrode impedance 8–10 kΩ at 1 kHz). One of these bipolar electrodes was implanted in the DRN (stereotaxic coordinates AP: -8.0, L: 0, DV: 6.5 mm from bregma and dura), the other in the optic chiasm (AP: -1.4, L: 1.0, DV: 9–9.5 mm from bregma and dura) (Paxinos & Watson, 1986). Electrode locations were later identified in 30-μm cresyl violet stain coated serial coronal sections. All electrodes were located in the target areas with the exception of one rat whose data were discharded.

The epidural EEG was monitored using 0.8-mm screws placed in the frontal and parietal bone. The ERG was recorded using multistrand, teflon-coated, stainless steel wire (Medwire) located under the upper eyelid. A stainless steel plate (3 $\times$ 5 mm) inserted under the skin over the masseter muscle served as a reference electrode. The ERG potentials were elicited by a red light–emitting diode glued to the skull above the eyeball, which provided retrobulbar stimuli (duration: 2 ms; luminance: 4.37 cd/m$^2\times$s; wavelength: 651 nm). Experiments began at least 10 days after implantation, with the rat placed in a Plexiglas box (50 $\times$ 35 $\times$ 50 cm), where it was free to move. Before recordings begun, animals were kept in darkness for at least 30 minutes for a complete dark adaptation. All stimuli were applied when the rats were in a state of quiet wakefulness according to Gottesmann's criteria (Gottesmann, 1992).

All electrical signals were amplified by a Grass 8–10 B amplifier. Analogue to digital conversion was performed at a sampling rate of 5 kHz using a CED micro 1401 (Cambridge Electronic Design Ltd). Data were then stored on a PC hard disk for off-line analysis using signal 1.9 software (Cambridge Electronic Design Ltd). For ERG acquisition, each trial represented the average of 50 consecutive sweeps. The latency of the b-wave was defined as the time between the stimulus and the first positive deflection following the a-wave. The b-wave amplitude was measured from the trough of the a-wave to the peak of the b-wave. For the analysis of OPs, the ERGs were high-pass filtered above 40 Hz. OP amplitudes were measured peak to peak (i.e., from the preceding trough). All data are expressed as mean $\pm$ *SEM* (standard error of mean).

RESULTS

In order to demonstrate the presence of a functional pathway projecting from the DRN to the retina we first measured the response of the optic chiasm to

weak DRN stimulation (square pulses with an amplitude of 0.82 mA and a duration of 0.1 ms, elicited at 0.1 Hz). These stimuli led to a clear and robust response in the optic chiasm which exhibited an onset latency of 7.60 $\pm$ 1.64 ms, a peak latency of 9.23 $\pm$ 1.82 ms, and a mean amplitude of 43.98 $\pm$ 16.87 μV (n = 1000 trials; five rats) (Figure 1). The activity was highly localized: it disappeared when the stimulating electrode was moved more than 0.8 mm above or below the DRN or when the bipolar recording pair was lifted more than 0.4 mm above the optic chiasm. In addition, when the stimulus polarity was inverted the responses failed to reverse in polarity thus eliminating the possibility of volume conduction from some distant source. Taken together these findings indicate that the responses in the optic chiasm represent DRN axon activity that is about to enter the retina and therefore confirm the presence of an operational DRN to retina pathway.

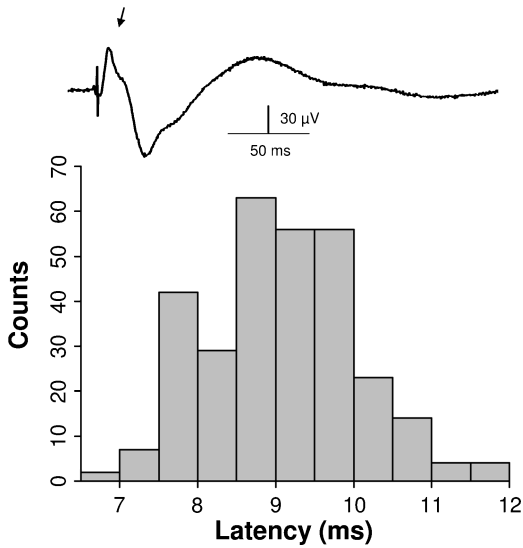

Figure 1. Averaged responses (n = 300 trials) from the optic chiasm after brief (0.1 ms) electric stimuli (0.82 mA) delivered to the DRN. The latency distribution of the first positive component's peak latency (arrow) is shown in the histogram below

In a second set of experiments the effects of DRN stimulation on endogenous retinal processes were investigated. In order to do this we examined the ERG of freely moving rats in control conditions and then compared it with the ERG obtained following DRN stimulation (with the same parameters as above). For the latter, we systematically varied the time interval between the two stimuli (electrical and light) from 0 to 500 ms. In the control condition the latency of the b-wave (31.01 ± 1.64; $n = 25$ trials) and the peak latency of the second, third, and fourth OP (OP_2: 37.96 ± 0.96; OP_3: 43.73 ± 0.49; OP_4: 50.63 ± 0.49; $n = 25$ trials) of the ERG showed no significant difference ($p > 0.05$, student's t-test) between different days or different animals. However, following electrical stimuli of the DRN the properties of the ERG could be substantially modified. In particular, when the interval between the DRN stimulation and the light flash was in the range 200–500 ms, we observed a significant decrease in the latency of the b-wave ($p < 0.05$) (Table 1). A significant reduction in the peak latency of the associated OPs was also observed ($p < 0.05$) (Figure 2C, D). DRN stimulation had no effect on b-wave amplitude (Table 1). However, the amplitudes of the accompanying OPs were notably increased. This was the most prominent for the third and fourth OP, which showed statistically significant increases in amplitude for all stimulus intervals between 0 and 400 ms ($p < 0.05$) (Figure 2E). Following the cessation of DRN stimulation, all properties of the ERG were restored to control levels (Table 1) (Figure 2).

DISCUSSION

The main findings of this study are: (i) following stimulation of the DRN, a reliable electrical response is elicited in the optic chiasm which results from activation of DRN fibres projecting to the retina, (ii) for a specific range of intervals between DRN stimulation and the triggering of light-evoked potentials there is a significant decrease in the latency of the b-wave and the peak latency of the accompanying OPs of the ERG, and (iii) for nearly all stimulus intervals, activation of the DRN–retina pathway caused a pronounced increase in the amplitude of the OPs. Importantly, the effects of DRN stimulation on the peak latency and amplitude of OPs cannot be explained by light adaptation because the amplitude of the b-wave, which is acutely sensitive to such adaptation, remained constant throughout the recordings. Thus, this is the first study to (i) provide direct evidence for the presence of a functional projection from the DRN to the retina and (ii) examine the physiological significance of this projection for early stage visual processing.

Table 1. Effect of DRN stimulation on the latency and amplitude of the ERG b-wave. Data are expressed as mean ± *SEM* (n = 25 trials; 5 rats) (*$p < 0.05$)

DRN Stimulation to Flash Interval (ms)	Control	0	20	50	200	300	400	500	Recovery
B-Wave Latency (ms)	31.01 ± 1.64	29.18 ± 1.77	29.68 ± 1.54	28.96 ± 2.24	*28.59 ± 1.74	*28.37 ± 1.32	*28.05 ± 2.06	*27.83 ± 1.97	31.28 ± 1.27
B-Wave Amplitude (% of control)	100	102.61 ± 3.06	101.05 ± 13.53	95.99 ± 10.63	98.03 ± 15.99	109.96 ± 23.88	97.33 ± 21.13	93.97 ± 14.59	104.1 ± 7.37

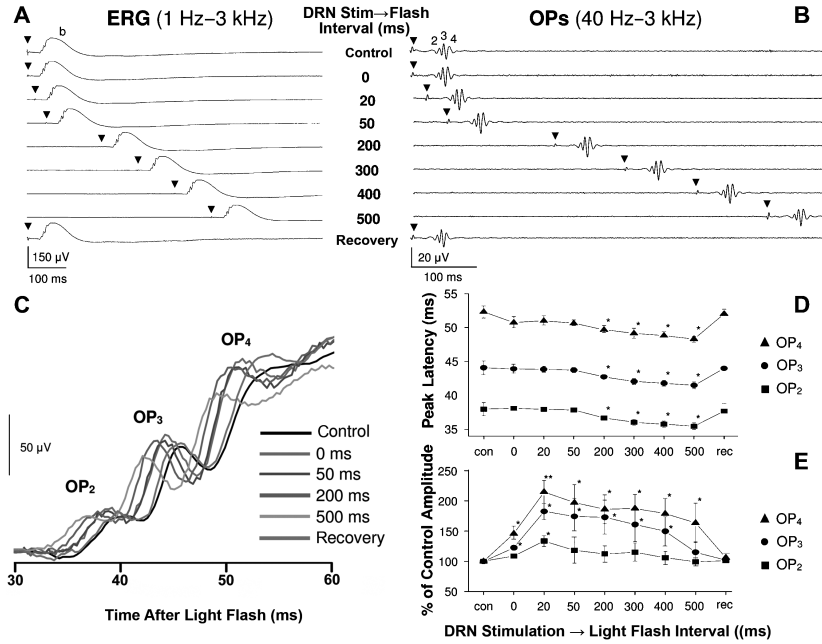

Figure 2. Effect of DRN stimulation on the ERG of freely moving rats (A and B) Averaged responses (n = 50) recorded from the cornea were evoked by 2-ms red light flashes (arrowheads) either in control conditions or following a 0.1 ms current pulse applied to the DRN. The interval between DRN stimulation and the light flash (DRN Stim → Flash Interval) varied from 0 to 500 ms. Representative examples of the effect of DRN stimulation are shown for the ERG b-wave (A) and OPs (B). (C) Initial segments of selected responses from (A) on a faster time base. (D) Plot of peak latency for the second (OP_2), third (OP_3), and fourth (OP_4) OPs of the ERG vs. stimulus interval duration (mean ± *SEM*) (n = 25 trials for each point; five rats). (E) Plot of peak to peak amplitude for the second (OP_2), third (OP_3), and fourth (OP_4) OPs of the ERG vs. stimulus interval duration (mean ± SEM) (n = 25 trials for each point; five rats) ($^*p < 0.05$, $^{**}p < 0.01$).

While both the latency of the b-wave and the peak latency of the associated OPs could be significantly decreased following DRN stimulation, this effect was most prominent for the OPs (see Table 1 and Figure 2D). In addition, the amplitude of the OPs could be subject to a DRN-induced increase, whereas the magnitude of the b-wave never was. Since OPs are thought to be generated by third order neurons in the inner retina (Dong, Agey, & Hare, 2004), whereas the b-wave derives, at least in part, from depolarization through a K^+ current loop between bipolar and Müller cells (Stockton & Slaughter, 1989), these different response profiles for the b-wave and OPs are clearly consistent with previous anatomical findings showing that retinopetal fibers terminate in the vicinity of

amacrine cells (Polyak, 1941). However, the precise spatial segregation and intraretinal localization of the retinopetal fiber system originating from the DRN clearly needs to be further clarified. In connection with this, since at least four different serotonin receptors types have been identified in the retina (Perez-Leon, Sarabia, Miledi, & Garcia-Alcocer, 2004; Pootanakit & Brunken, 2000, 2001; Pootanakit, Prior, Hunter, & Brunken, 1999), another key issue for further studies will be to determine which of these are responsible for mediating the effects of the DRN input.

As the ERG can be subject to a pronounced modification in response to DRN stimulation, it seems reasonable to suggest that during states of enhanced vigilance, when DRN neurons fire more intensely than during slow wave and REM sleep, this structure might be able to exert an enhanced influence over retinal processing. Interestingly, we recently showed that flash-evoked ganglion cell responses in freely moving rats vary systematically between different stages of arousal (Galambos et al., 2001). Specifically, this study showed that while the ERG b-wave amplitude remained constant, the light-evoked potentials recorded from the optic chiasm, which presumably reflect ganglion cell population activity (Galambos et al., 2000), were increased during slow wave and REM sleep compared to wakefulness (Galambos et al., 2001). We proposed that differential activity in the DRN–retina pathway might account for these findings. Clearly, the invariance of the b-wave amplitude with respect to wakefulness and sleep is consistent with the finding in this study that the b-wave amplitude is unaltered by DRN stimulation. In addition, the alterations in OP amplitudes following DRN stimulation could be considered coherent with a change in the presumed ganglion cell output observed between wakefulness and sleep. Unfortunately, it was not possible in this study to investigate how changes in the ERG OPs induced by DRN stimulation relate to changes in ganglion cell population responses, because our electrical recordings from the optic chiasm were obviously contaminated by the DRN stimuli (see Figure 1). However, because we did not see any consistent changes in OPs between wakefulness and sleep in the present investigation, it appears unlikely that changes in the activity of the DRN pathway can solely explain the apparent alterations in ganglion cell population output that occur in response to arousal state changes.

Further experiments are clearly required to determine the precise physiological circumstances under which the DRN most prominently affects retinal processing. Nevertheless, this study firmly establishes that such effects can directly take place through the retinopetal fiber system. We propose that the specific role of the DRN in this respect may be to improve the temporal

sharpness and efficiency of retinal responsiveness, enabling it to act as a "fine tuning" device for early stage visual processing (Eason, Oakley, & Flowers, 1983).

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