Serotonin-Caused Phase Shift of Circadian Rhythmicity in a Photosensitive Neuron

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KEY WORDS crayfish; Procambarus clarkii; Cherax quadricarinatus; sixth abdominal ganglion; caudal photoreceptor; 5-hydroxytryptamine; 5-HT; 8-OH-DPAT; 5-HT1A receptor

ABSTRACT In the sixth abdominal ganglion (sixth AG) of the crayfish, two photosensitive neurons are located and have been identified as caudal photoreceptors (CPRs). We have expanded our investigation on the role of 5-Hydroxytryptamine (5-HT) as a modulator of the spontaneous and light-induced activity of the CPR. We located, by using immunocytochemistry, neurons in the sixth AG that contain the 5HT1A receptor. The expression of these receptors was examined by binding assays with [3H] 8-hydroxy-2 (di-n-propylamino) tetralin ([3H(8-OH-DPAT). We examined the exogenous action of both 5HT and its agonist 8-OH-DPAT on the phase of circadian rhythms of the spontaneous electrical activity and the photoresponse of the CPR in the isolated sixth AG by conventional extracellular recording methods. Experiments were made on the adult crayfish Procambarus clarkii and Cherax quadricarinatus. Thirteen immunopositive neurons were located, principally near the ventral and dorsal surface of the sixth AG, with the mean diameter of their somata 20 ± 3 μm. The specific binding data showed the presence of 5-HT1A receptors with a mean level of 22.4 ± 6.6 fmol/mg of wet tissue. Spontaneous and light-induced electrical activity of the CPR showed circadian variations with their activity more intense at night than in the day. Exogenous application of 5-HT or 8-OH-DPAT causes a circadian phase-shift in electrical activity of the CPR. Taken together, these results lead us to believe the 5-HT acts as a modulator of circadian electrical activity of the CPR in the isolated sixth AG of crayfish. Moreover, it suggests that the 5-HT1A receptor participates in this modulation. Synapse 61:801–808, 2007. © 2007 Wiley-Liss, Inc.

INTRODUCTION Extraretinal photoreceptors are present in both vertebrates and invertebrates (Kartelija et al., 2003; Vigh et al., 2002). In the crayfish, the sixth abdominal ganglion (sixth AG) contains two photosensitive neurons. These are on the anterior part of the ventral side of the ganglion (Prosser, 1934; Welsh, 1934; Wilkens and Larimer, 1972, 1976). Their physiological properties and role in the behavior of the animals have been extensively studied (Larimer and Moore, 2003; Rodriguez-Sosa et al., 2006; Wilkens, 1988). This caudal photoreceptor (CPR) displays spontaneous electrical activity and phasic-tonic responses to light pulses. It has been reported that these spontaneous and light-caused firing rates of the CPR show circadian oscillations (Prieto-Sagredo and Fanjul-Moles, 2001; Rodríguez-Sosa et al., 2003; Sosa and García, 2000). Additionally, it has been postulated that these photoreceptors participate in the synchronization of circadian rhythms (Bernal-Moreno et al., 1996; Fuentes-Pardo and Inclán-Rubio, 1987).

Serotonin (5-Hydroxytryptamine, 5-HT) has been postulated as a modulator of the circadian oscillator in vertebrates and invertebrates (Guillete et al., 1993; Prosser, 2003; Saifullah and Tomioka, 2002; Steven...
and Jacklet, 1997). In the mammalian suprachiasmic nucleus, the effect of the 5-HT and its agonist causes resets of this circadian clock in vitro and in vivo on the patterns of drinking and running in different animals (Medanic and Guillette, 1992; Prosser, 2003). The 8-hydroxy-2 (di-n-propylamino) tetralin (8-OH-DPAT), a specific agonist of the 5-HT1A receptor, causes a phase advance of behavioral rhythm and the spontaneous neuronal activity in vitro (Horikawa and Shibata, 2004; Prosser et al., 1993). In crustaceans, there is an evident correlation between the diurnal variation of this amine in their central nervous system (Castañón-Cervantes et al., 1999; Escamilla-Chimal et al., 1998; Fingerman and Fingerman, 1977; Wildt et al., 2004) and their motor and sensory activity (Aréchiga and Rodríguez-Sosa, 1997, 2002). More recently, we have suggested that electrical activity of the CPR is modulated by serotonin because we have observed a pool of serotonin-immunopositive neurons in the sixth AG. In this ganglion, the content of serotonin and its precursors has diurnal variations (Table I). Additionally, exogenous serotonin enhances the basal activity of the CPR in the dark and reduces its response to light in a dose-dependent manner (ED50 ~ 1 μM), with both effects blocked by methysergide (Rodríguez-Sosa et al., 2006). We have also observed a diurnal rhythm in the levels of the 5-HT1A receptors in the crayfish eyestalk (ES) (Calderón-Rosete et al., 2006). However, little is known about the influence of this biogenic amine on the circadian rhythmicity of the CPR (Rodríguez-Sosa et al., 2005).

These data cited above suggest that 5-HT is a modulator of the circadian rhythm of the CPR and that 5-HT1A receptors may be part of this circadian system of the caudal photoreceptor in crayfish. In our work, we have explored the role of serotonin as a possible modulator of the circadian electrical activity in the crayfish CPR. Our aims are (a) to study the immunolocalization of the neurons in the sixth AG that contain the 5-HT1A receptor, (b) to determine specific binding sites using ([3H]8-OH-DPAT), an agonist of the 5-HT1A receptor, by an autoradiography procedure in the sixth AG, and (c) to test the effect of external 5-HT and the specific agonist 8-OH-DPAT on the circadian rhythms of the spontaneous electrical activity and the photoresponse of CPR in the isolated sixth AG.

**MATERIALS AND METHODS**

**Animals**

Experiments were made on adult crayfish Procambarus clarkii and Cherax quadricarinatus of either gender and in intermolt. The specimens were collected in field trips or purchased from breeding facilities in México. The crayfish were kept in the laboratory for 2 weeks before experimentation in controlled

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**Table I. Rhythmic changes in the sixth AG of the crayfish (Procambarus clarkii and Cherax quadricarinatus) in the 24-h cycle**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Amplitude (Clock time)</th>
<th>Rhythm (%)</th>
<th>ANOVA F-test*</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT content b,c</td>
<td>50.4 (fmol/mg protein)</td>
<td>22.4 (fmol/mg protein)</td>
<td>1024 91</td>
</tr>
<tr>
<td>5-OH-TRP content b,c</td>
<td>7.6 (fmol/mg protein)</td>
<td>5.9 (fmol/mg protein)</td>
<td>1100 98</td>
</tr>
<tr>
<td>L-TRP content b,c</td>
<td>1.3 (pmol/mg protein)</td>
<td>0.42 (pmol/mg protein)</td>
<td>2318 74</td>
</tr>
<tr>
<td>Spontaneous activity of the CPR d</td>
<td>0.74 (normalized data)</td>
<td>0.10 (normalized data)</td>
<td>1942 40</td>
</tr>
<tr>
<td>Photoresponse of the CPR d</td>
<td>0.62 (normalized data)</td>
<td>0.26 (normalized data)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

*Values in parentheses indicate P values. (See text).

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12:12 light:dark cycles (LD). All procedures described in this study are in accordance with the “Guide for the Care and Use of Laboratory Animals” of the Mexican Council for Animal Care as approved by the UNAM Animal Care Committee. All efforts were made to minimize animal suffering and to reduce the number of animals used.

**Immunocytochemistry**

Three isolated sixth AG from *Procambarus clarkii* were fixed for 3 h at 4°C in 4% paraformaldehyde, 0.25% picric acid, diluted in 0.1 M phosphate-buffer saline solution (PBS) at pH 7.4. Sections of the tissue (16 μm) were obtained with a cryomicrotome (IEC/Minitome Plus), collected on gelatin-coated slides, and stored at −20°C until immunoassay. To saturate non-specific sites, the sections were incubated 20 min at room temperature in 0.1 M PBS, pH 7.4, containing 0.03% Triton X-100 (TX100 Sigma) and 4% normal goat serum (NGS). After rinsing the slides with PBS, the sections were then incubated for 48 h at 4°C with the primary antibody, guinea pig antiserosotonin receptor 1A (5-HT1A) polyclonal antibody (Chemicon International), diluted 1:200 in PBS containing 0.03% TX100 and 4% NGS. Sections then were washed with PBS and incubated for 1 h with Texas Red anti-guinea IgG secondary antibody (Vector Laboratories). After rinsing the slides with PBS, the sections were then incubated for 48 h at 4°C with the primary antibody, guinea pig antiserosotonin receptor 1A (5-HT1A) polyclonal antibody (Chemicon International), diluted 1:200 in PBS containing 0.03% TX100 and 4% NGS. Sections then were washed with PBS and incubated for 1 h with Texas Red anti-guinea IgG secondary antibody (Vector Laboratories). After rinsing with PBS, slides were covered with VectaShield mounting medium (Vector Laboratories). Photomicrographs of histochemical preparations were captured and analyzed using a computerized image-analysis system (MetaMorph V. 4.5, Universal Imaging Corporation, PA) coupled to a fluorescence microscope (Nikon) equipped with the filters appropriate for the fluorochrome mentioned. For control staining, one out of five slices was placed on a slide apart and anti-5HT 1A antibody was not added to the incubation solution.

**Autoradiography**

The sixth abdominal ganglia from 10 crayfish *Procambarus clarkii* and from one *Cherax quadricarinatus* were dissected in a saline solution (modified from Van Harreveld, 1936) consisting of (in mM) 205 NaCl, 5.4 KCl, 2.6 MgCl2, 13.5 CaCl2, and 10 HEPES-Na with pH adjusted to 7.4, at 4°C. After dissection, the sixth AGs were immediately frozen in dry ice and stored at −70°C until use. The ganglia were sectioned into 16-μm slices on the longitudinal plane by using a Leica CM1100 cryostat at −20°C. Sections were collected on clean, gelatin-coated microscope slides and stored at −70°C until the day of the experiment. All tissue sections were processed at the same time. Slide-mounted tissue sections were incubated in 50 mM TRIS, pH 7.4, for 15 min at room temperature (RT). Sections were then incubated with 2 nM of [3H]-8-OH-DPAT for 60 min at RT. Incubations were ended by dipping the slices into ice-cold buffer followed by four consecutive 2-min washes in the same buffer. After a final dip in ice-cold distilled water, the slices were dried in a stream of cold air. Nonspecific binding was determined in the presence of 5-HT (10 μM). Sections were apposed to [3H]-Hyperfilm for 8 weeks at RT alongside microscale-calibrated tritium standards.

For data analysis, the films from the autoradiography assays were analyzed using a computerized image-analysis system (MCID System, Imaging Research Inc, St. Catherine, Ontario, Canada). Central regions of the sixth AG were selected. The mean optical density was determined by measurements from four sections of the same sixth AG for specific binding. The nonspecific binding was determined in four adjacent sections as well. The mean optical density of specific binding minus the mean optical density for nonspecific binding is the value for each sixth AG. The mean binding (± SE) data are expressed as fmol/mg wet tissue.

**Electrophysiology**

The abdominal ganglionic chain was removed from the experimental animals under ice-cold modified saline solution of Van Harreveld. The sixth AG was excised and pinned down in a recording chamber, either under saline solution or under organ-culture medium, as described earlier (Aréchiga and Rodríguez-Sosa, 1998) with 20% Leibovitz L-15 Medium, 5% fetal calf serum, and an antibiotic mixture of penicillin-amphotericin-gentamicin. The pH was adjusted to 7.4 with HEPES-Na. All solutions were sterile filtered using a Millipore (0.22 μm) filter. Preparations in culture were maintained at 16°C. After dissecting the sixth AG, the nerve cords were cut near the fifth ganglion and then dissected between the fifth and the sixth interganglionic connective tissues to expose the nerve bundles. The preparation was transferred to a petri dish containing modified Van Harreveld saline solution and then pinned down in the recording double-bottom chamber mounted on a microscope (SMZ800, Nikon) and perfused with saline solution (about 1 ml/min) for 1 h in darkness at 16°C before beginning the recording. Preparations were kept in a temperature-regulated chamber with the temperature maintained by the recycled pumping of cool water (Cole Parmer). The extracellular recordings were made with suction electrodes prepared by polishing glass micropipettes and filling them with saline solution. The suction electrode was usually positioned on the distal part of the connective tract. The reference electrode (Ag-AgCl) was connected via an agar bridge to the bath solution. Signals were recorded with AC amplifiers (EX1, Dagan) filtered at 30 Hz to 10 kHz.
by a band-pass filter system and displayed on an oscilloscope (Gould 1604). One-second duration light pulses usually at 60-min intervals were delivered with a Grass photo stimulator (PS33), which produced white incandescent light (1000 lux). Light intensity was attenuated with neutral density filters (Kodak, series No. 96). Calibration of intensities was done with a photographic lightmeter (Goossen, model Luna-Pro, Germany). Usually, in this study the preparation was stimulated with intensities of 60 lux. The electrophysiological signals (Fig. 1) were captured at 20 kHz on a computer using the program Spike2 (V. 5, Cambridge Electronic Designed. Cambridge, England) and analyzed off-line with this software.

Chemicals

In our preparations we tested the effect of 5-HT (10 \( \mu M \)) (Sigma) or the agonist 8-OH-DPAT (10 \( \mu M \)) (Sigma) by incubating them in the isolated sixth AG for 1 h. Then, the sixth AG was washed in culture medium three times and kept in this medium at constant temperature (16°C). Control preparations were incubated only with modified Van Harreveld solution and were also maintained in the same isolated culture conditions. Electrical activity usually was recorded about 24 h later.

Statistical analysis

The firing rate of spikes (Hz) from CPR was normalized and then a circadian-rhythm analysis was made for the conventional parameters; mesor, amplitude, acrophase (with a fundamental period manually varied from 20 to 28 h in 1-h steps until getting the best fit). This work is based on the partial Fourier analysis. An analysis was also made to determine if the nonlinear regression curve was statistically significant by a variance analysis with an \( F \)-test at a probability of \( P < 0.05 \). The proportion of variance (\( R^2 \)) explained by this model was referred to as rhythm percentage (Rodríguez-Sosa et al., 2006). This was

Fig. 1. A: A scheme of the crustacean. In the nervous system, the sixth AG is inside the square. B: A scheme of an extracellular electrode array to record the activity from the sixth AG. The approximate location of a transversal cut axon of the CPR (arrow) in the connective tissue between the sixth and fifth ganglion is also represented. In (C), electrical activity caused by light (60 lux for 1 s). Below, electrophysiological records with firing rates (in Hz) shown. Scale bar is indicated.

Fig. 2. Immunopositive neurons in the sixth AG of the crayfish *Procambarus clarkii* stained with an anti-5HT1A receptor antibody. Position of the somata and axons are indicated on diagram. (A) anterior, (P) posterior, (D) dorsal, and (V) ventral. Digital micrographs of representative cells near the dorsal, medial, and ventral region are indicated by lines A, B, and C. Scale bar of 20 \( \mu m \) is indicated.
made with the software Chronos-Fit (V1.05) developed by Zuther and Lemmer (2005).

RESULTS

Immunolocation of the 5-HT$_{1A}$ receptors in the sixth AG

In the sixth AG of Procambarus clarkii, immunoreactive 5-HT$_{1A}$ (5-HT$_{1A}$-ir) was observed in the cytoplasm of a group of 13 immunopositive neurons, with the mean somata (± SE) diameter of 20 ± 3 μm. These somata are connected through 5-HT$_{1A}$-ir in axons and appear to project to neuropil. They were located in three preparations, principally near the ventral and dorsal surface of the sixth AG (Fig. 2). Additionally, in the connective tissue between the sixth AG and fifth AG, a longitudinal coiled-like fiber was observed. As a control in the two ganglion immunohistologys no primary antibody (anti-5HT$_{1A}$) was added and there was no staining of neurons or neuropilar fibers (not shown).

5-HT$_{1A}$ receptor autoradiography in the sixth AG

In Figure 3A, representative autoradiograms for the tritiated agonist to the 5-HT$_{1A}$ receptor for specific and nonspecific binding in the sixth AG are illustrated. The specific binding for the 5-HT$_{1A}$ receptor in the sixth AG had a mean level of 22.4 (± 6.6) fmol/mg of wet tissue for Procambarus clarkii. The Cherax quadricarinatus yielded similar data (16.7 fmol/mg of wet tissue) (Fig. 3B).

Neuronal firing rate of CPR during a 24-h cycle

As described earlier by the authors (Wilkens and Larimer, 1972, 1976) in several crayfish, the isolated sixth ganglion CPR has a spontaneously discharges in the dark with a firing rate (mean ± SE) of 4 ± 1.6 Hz (n = 72). The spontaneous activity in the dark also varied in a circadian manner. A low spontaneous activity in the day and an increase in the nocturnal phase were measured (Fig. 4). A Chronos-Fit model applied to the spontaneous activity for averaged data per hour (clock time) of three CPRs showed a circadian rhythm. The period was fixed at 25 h, an acrophase at clock-time 1942, amplitude 0.10 (normalized spontaneous firing rate), mesor 0.74 (normalized spontaneous firing rate), and rhythmicity of 40%,
The CPR response to test light-pulses was a sustained train of spikes (Figs. 1C and 1D). The photoresponse shows a long latency (1.8 s) and continues all through the illumination period and several seconds after light is off. Beyond a certain level of intensity (30 lux), a phasic initial peak of activity ensued, which gradually decreased until reaching a plateau. The firing rate of spikes both at the peak and the plateau is a function of light intensity. As seen in Figure 4, light-caused discharges of the CPR vary along the 24-h cycle, with a low light-responsiveness in the day and a nocturnal maximum response. The Chronos-Fit model applied to the firing rate of spikes at the peak of averaged data per hour (clock time) from three preparations showed a rhythm with a 24-h fixed period. The acrophase was at 0106, amplitude 0.26 (normalized photoresponse), mesor 0.62 (normalized photoresponse), and rhythmicity of 86%, $F_{2, 24} = 67.3$, which was statistically significant ($P < 0.001$).

**Phase-shifting of the rhythms of the CPR by serotonin and by 8-OH-DPAT**

Another feature of circadian rhythms is that environmental synchronizer agents can entrain them. In our preparations obtained from Cherax quadriracarina-tus, we tested the effect of 5-HT (10 $\mu$M) or the agonist 8-OH-DPAT (10 $\mu$M). The control preparations were incubated only with modified Van Harreveld solution (see methods). The spontaneous activity rhythm from the CPR recorded in vitro had a forward shift in the acrophase of ($\text{mean } \pm \text{SE}$) 5.3 $\pm$ 0.7 h ($n = 5$). For the photoresponse, a delay of the acrophase was measured with a shift of 4.2 $\pm$ 1.2 h ($n = 5$). These phenomena took place after an application of serotonin at 1300. Similar data were obtained with its agonist 8-OH-DPAT, shown in Figure 5.

**DISCUSSION**

In our report, modulation of the circadian electrical activity of the CPR by serotonin in the isolated sixth AG of crayfish is explored. We suggest that this activity of the CPR is also regulated by a probable circadian rhythm of serotonin, originating in its own sixth AG. We also discuss the functional organization of this serotonergic modulation.

We have previously suggested that, depending on the time of day, endogenous 5-HT may influence crustacean behavior by modulating the electrical activity of the caudal photoreceptor neurons (Rodrı́guez-Sosa et al., 2006). This concept merges with our more recent observation that the content of 5-HT in the sixth AG displays a diurnal variation with the acrophase at midday (Table I). We have also suggested a possible circadian rhythm of the release of 5-HT from 5-HT-ir neurons in the sixth AG (Rodrı́guez-Sosa et al., 2006). Recently, Wildt et al. (2004) have shown that in the brain of the lobster Homarus americanus serotonin levels are controlled by an endogenous clock and regulated by light. In addition, one of our previous reports suggests a seasonal rhythm of the 5-HT content in the crayfish ES (Caldero´ n-Rosete et al., 2001).

The number of 5-HT$_{1A}$-ir neurons reported in our work matches that of the 5-HT-immunopositive somata, which we have previously described (Rodrı́guez-Sosa et al., 2006), and the number is just incidental because these neurons are located at different sites and have different dimensions. The distribution of 5-HT$_{1A}$-ir neurons in the sixth AG shows a pattern that resembles the one reported by Spitzer et al. (2005) in the crayfish. We usually found one to three cell bodies on the dorsal surface at the lateral anterior and posterior margin (Fig. 2), whereas Spitzer et al. (2005) had observed somata clusters. These authors note that the expression of 5-HT$_{1crust}$ in the nerve cord of crayfish is highly variable among animals, with a few preparations showing high numbers of labeled somata. Identification of the neurons by immunostaining and autoradiographic techniques shows different sites of specific binding. The 5-HT$_{1A}$ polyclonal antibody employed in our work recognizes the third intracellular loop of the 5-HT$_{1A}$ receptor (Pompeiano et al., 1992). Spitzer et al. (2005) have used an antibody (anti-5-HT$_{1crust}$) against two representative peptides conserved in the 5-HT receptors of the crayfish, lobster, and prawn (Sosa et al., 2004). The 5-HT$_{1A}$
receptor is the most studied receptor among the serotonin receptors. This is in part because of the availability of the selective ligand 8-OH-DPAT and because this highly selective prototypical agonist has high potency in vivo and has high affinity (Kd = 0.3–1.8 nM) for the receptor isolated from various sources (Pucadyil et al., 2005). In our work [3H]-8-OH-DPAT was used at a concentration of 2 nM in the autoradiographic technique, the same used in specific binding in vertebrates (Chalmers and Watson, 1991). Our contribution using the autoradiography method shows the presence of 5-HT1A receptors in the sixth AG. It has been proposed that the expression of the levels of 5-HT1A receptors is modulated by LD cycles, as observed in the crayfish ES (Calderón-Roseste et al., 2006).

Once the presence of the 5HT1A receptors in the sixth AG by immunostaining and autoradiographic techniques was characterized, we contributed with a study of 5-HT and their agonist 8-OH-DPAT on the electric activity of a neuron identified in the nervous system of crayfish. The CPR has a tonic endogenous pacemaker-like electrical-activity pattern (Larimer, 2000). As noted in the introduction, spontaneous and light-induced electrical activity of the CPR revealed circadian variations, with the activities being more intense at night than during the day (Fig. 4, Table I). We have also noted that topical application of serotonin to isolated ganglia kept in the dark increases the spontaneous firing rate of the CPR (by a possible depolarization of the membrane) and reduces its light response (Rodríguez-Sosa et al., 2006). Our results suggest for first time that 5-HT and 8-OH-DPAT act as modulators of circadian electrical activity of the CPR in the isolated sixth AG of crayfish (Fig. 5). The data obtained show that the CPR in the isolated sixth AG is sensitive to a phase shift caused by the effect of serotonin and 8-OH-DPAT. We suggest this effect is mediated by the 5-HT1A receptor. From data reported in several vertebrates, our results also show an advance of the circadian electrical-activity phase of the CPR for the exogenous application of 5-HT or 8-OH-DPAT (when applying them during the subjective photo-phase). As has been observed for the recordings of the spontaneous electrical activity in vitro of the supra-chiasmatic nucleus and in other behavioral patterns in a variety of animals, this activity yields phase shifts with the application of 5-HT or its agonist 8-OH-DPAT (Edgar et al., 1993; Horikawa and Shibata, 2004; Prosser, 2003; Prosser et al., 1993). In addition, our current data discloses that serotonin or 8-OH-DPAT causes an advance in the spontaneous activity acrophase whereas it produces a delay in the photosensitive phase (Fig. 5). These results suggest a complex mechanism of control by serotonin or its agonist 8-OH-DPAT in both circadian rhythms of the CPR (Morin and Allen, 2006). This points to further studies being needed to confirm this hypothesis, i.e., to assess the effect on the extraretinal neurons of the 5-HT1A agonist receptor 8-OH-DPAT at different times of the day.

Additionally, these data are in a close connection with the work of Arechiga and Rodríguez-Sosa (1998, 2002) where they suggested that the central nervous system of the crustacean appears to harbor discrete sets of independent circadian pacemakers, a property also observed in other animals (Menaker, 2006). These mechanisms act coordinately in the sixth AG for the expression of both rhythmicity and modulation in the CPR (Table I) in which expression contributes to maintain the proper relationship between morning and evening activity and their functions, as has been postulated for many biological processes (Guillete and Sejnowski, 2005; Menaker, 2006; Stoleru et al., 2005).

To conclude, we summarize the following:

(1) In the sixth AG there are 13 immunopositive 5-HT1A receptor neurons with somata mean diameter of 20 ± 3 μm. (2) The specific binding data show the presence of the 5-HT1A receptors in the sixth AG. (3) Spontaneous and light-induced electrical activity of the CPR revealed circadian variations, both being more intense during the night than in the day. (4) Serotonin or its agonist 8-OH-DPAT causes a phase shift in the circadian rhythms of spontaneous activity and the responsiveness of this activity to light. (5) Taken together, these results lead us to believe that the 5-HT acts as modulator of circadian electrical activity of the CPR in the isolated sixth AG of crayfish. (6) The CPR is sensitive to the in vitro phase resetting by serotonin and the 5-HT1A receptor probably participates in this modulation.

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