Circadian rhythms of dopamine, glutamate and GABA in the striatum and nucleus accumbens of the awake rat: modulation by light

Abstract: Using microdialysis, we investigated the circadian rhythms of the extracellular concentrations of dopamine, glutamate and γ-aminobutyric acid (GABA) in the striatum and nucleus accumbens of the awake rat. Wistar rats were maintained in a 12 hr dark:12 hr light (12:12) cycle for 2 wk before the experiment began. The neurotransmitter levels were measured every 30 min for 30 hr in control (maintaining the 12:12 cycle) or in experimental conditions under a 24-h light period (continuous light) or under a 24-h dark interval (continuous dark). The dopamine metabolites, DOPAC and HVA, and the main serotonin metabolite, 5-HIAA, were measured along with arginine and glutamine under all conditions. In 12:12 conditions, a circadian rhythm of dopamine, glutamate and GABA was found in both the striatum and nucleus accumbens. Again under 12:12 conditions, DOPAC, HVA, 5-HIAA, and arginine, but not glutamine, fluctuated in a circadian rhythm. In the striatum under constant light conditions, there was a circadian rhythm of dopamine, glutamate, GABA, DOPAC and HVA, but not 5-HIAA. By contrast, when the rats were kept under continuous dark, dopamine and its metabolites, DOPAC and HVA (but not glutamate and GABA), did not fluctuate in a circadian rhythm. In the nucleus accumbens, under both constant light or dark conditions, no changes were found in the circadian rhythm in any of the neurotransmitters and metabolites studied. These findings show that in the striatum, dopamine but not glutamate and GABA, seem to be influenced by light. In the nucleus accumbens, however, the three neurotransmitters had a circadian rhythm, which was independent of light.

Introduction

The striatum and nucleus accumbens are two brain areas whose functions are related to motor, emotion and motivation [1]. However, very little is known about the possible control of the circadian oscillations of neural circuits within these areas mediated by specific neurotransmitters in the awake animal. Several studies have reported circadian changes in extracellular concentrations of some neurotransmitters, particularly the extracellular concentrations of dopamine (DA) and its metabolites [2–5] and of serotonin and its metabolites [6] in the striatum and nucleus accumbens. In addition, noradrenaline in the pineal gland [7] and acetylcholine in different cortical areas have a circadian variation [8–10]. Glutamate (GLU) and γ-aminobutyric acid (GABA) are two of the most abundant neurotransmitters in the central nervous system [11, 12], but despite their importance in many functions of the brain [13–16], there are very few reports on possible circadian variations of these neurotransmitters in the awake animal [13]. We recently reported circadian variations of GLU and GABA and the effects of melatonin on these circadian changes in the striatum [17].

The striatum receives glutamatergic afferents from the cortex by the cortico-striatal pathway and a dopaminergic projection from the substantia nigra pars compacta through the nigro-striatal pathway [18]. The striatum also contains intrinsic as well as projecting GABA neurones. The nucleus accumbens is also an area of convergent terminals containing DA and GLU. DA in the nucleus accumbens is released from terminals of neurones located in the ventral tegmental area [18]. Glutamatergic terminals originate from neurones located in different areas such as the prefrontal cortex, hippocampus and amygdala [18]. As in the case of the striatum, the nucleus accumbens contains intrinsic GABA neurones.

It would be interesting to determine the possible circadian changes of these three neurotransmitters, DA, GLU and GABA, in the two areas of the brain and also whether they are influenced by changes of the dark–light cycle. In fact, the raphe nucleus receives direct inputs from the retina [19], and both the striatum and nucleus accumbens receive inputs from this nucleus. The existence of a night–day cycle in extracellular concentrations of serotonin has been reported [6] as well as in its main metabolite (5-HIAA) in the striatum [3, 4, 6] and nucleus accumbens [3, 4]. Given
the interaction, in the striatum and nucleus accumbens, between serotonin and DA [20–22] and between DA, GLU and GABA [23–25], it is possible that the neurotransmitters DA, GLU and GABA come under the direct control of a circadian oscillator and/or under the influence of light. In order to elucidate these possibilities we also studied the variations of these neurotransmitters under modified dark/light cycles.

The aim of this research was to investigate the possible circadian variations of the extracellular concentrations of DA, GLU and GABA in the striatum and nucleus accumbens in control situations, in a 12 h dark/12 h light cycle, and also under constant light or darkness conditions. The DA metabolites, DOPAC and HVA, and the main serotonin metabolite, 5-HIAA, were also measured, along with arginine (ARG) and glutamine (GLN). This design provides information on different neurotransmitters in specific circuits of the brain during the 24-h cycle and also on whether there are changes in the circadian rhythm of some of these neurotransmitters under physiological modifications such as constant periods of light or darkness. This research on whether specific circuits coding for specific neurotransmitters do oscillate during the day–night period would give considerable insight into our current knowledge of how the brain works.

Materials and methods

Animals and surgery

Male Wistar rats (2–4 months, 250–350 g weight) were housed in individual wire mesh cages, provided with food and water ad libitum, and maintained in a temperature-controlled room under a dark/light cycle (lights off/on at 8:00/20:00 hr). All in vivo experiments, performed at the Universidad Complutense of Madrid, followed the guidelines of the International Council for Laboratory Animal Science.

Under equithesin (2 mL/kg, i.p.) anaesthesia, bilateral guide-cannulae were stereotaxically implanted in the brain to accommodate microdialysis probes in the striatum of the rats. Guide-cannulae assembly [23] were then fixed to the skull by means of two anchorage screws and application of dental cement. When inserted, the tip of the microdialysis probe was placed into the striatum: 0.6 mm rostral, 2.5 mm lateral from bregma and 2.8 mm ventral from dura mater and the nucleus accumbens: 1.6 mm rostral, 1.6 mm lateral from bregma and 2.8 mm ventral from dura mater [26].

Microdialysis

Ten days after surgery, a microdialysis probe was inserted and the experiment was performed on the freely moving rat. Probes of concentric design were constructed in our own workshop with an active dialysing length of 4 mm for the striatum and 2.5 mm for the nucleus accumbens experiments [23]. The dialysis membrane had a molecular weight cut-off of 5000 Da (Hospal). The probes were perfused with artificial spinal fluid (composition in mM: NaCl, 137; CaCl2, 1.2; KCl, 3; MgSO4, 1; NaH2PO4, 0.5; Na2HPO4, 2; glucose, 3; pH 7.3) at a flow rate of 1.25 μL/min.

A. Control conditions

B. Light conditions

C. Dark conditions

![Fig. 1. Schematic diagram of the experimental design performed in these experiments: (A) control conditions, (B) light conditions, and (C) dark conditions.](image)

Once basal concentrations of catecholamines and amino acids were established, 30-min samples were collected for 30 hr (from 14.00 to 20.00 hr the next day) and immediately stored at −80°C until analysed. The control group was maintained under the same previous dark/light cycle (6 hr darkness/12 hr light/12 hr darkness) (Fig. 1A). The constant light group was maintained under 24 hr of light after the first 6 hr of darkness (6 hr darkness/24 hr light) (Fig. 1B) and the constant dark group was maintained under 24 hr of darkness after the first 6 hr of darkness (6 hr darkness/24 hr darkness) (Fig. 1C).

To test whether the animals had recovered their circadian rhythms when the experiments started, motor activity was measured 10 days postsurgery in the three studied conditions. The measurements were performed by photocell cages measuring 34 × 35 cm and containing 16 photocells and the results were analysed with the computer software Actim16x (Cibertec S.A., Madrid, Spain). The animals presented a circadian rhythm on the day of the experiments (P < 0.01) (data not shown).

The microdialysis system (tubing and swivel) was kept filled with a diluted solution of benzalkonium chloride (armil) between experiments to prevent bacterial growth. Finally the animals were anaesthetized with equithesin and perfused intracardially with 0.9% saline solution followed by 10% formalin. The brain was removed and the placement of the microdialysis probe was verified with a cryostat microtome and viewing lens.

Catecholamine analysis

The catecholamine content of the samples was analysed by reverse-phase high-performance liquid chromatography (HPLC) and electrochemical detection [23, 27, 28]. Samples were injected into a Rheodyne injector (20-μL loop) running first through a C18 precolumn (Nova-Pack; Waters Corporation, Milford, MA, USA) and then through a 3.9 × 150-mm C18 column of 4-μm particles (Nova-Pack). The mobile phase consisted of 0.1 M acetate-citrate buffer...
Amino acid analysis

The amino acid content of the samples was analysed by reverse-phase HPLC and fluorometric detection according to a method used previously in our laboratory [23, 27]. In brief, precolumn derivation of 5-μL samples was performed with o-phthalaldehyde solution. Derivatized samples were injected into a Rheodyne injector (20-μL loop) running through a Spherisorb® ODS-2 column (Waters Corp.). A gradient program of two mobile phases at a flow of 1 mL/min was used. Solution A was a 95:5 (v/v) mixture of 50 mM sodium acetate buffer (pH 5.65) and methanol, to which 12.5 mM/L isopropyl alcohol was added; solution B was a 70:30 (v/v) methanol/water mixture. These conditions allowed the amino acids to be detected within 15 min.

The amino acids were measured by a fluorescence detector (Waters 747). The excitation filter was set at 340 nm and the emission filter at 460 nm. Amino acids were quantified by using MAXIMA 820 (Waters Corp.) software by means of the internal standard procedure. The internal standard used was 0.05 mM homoserine. The detection limit in our 5-μL samples was 0.05 μM for all amino acids.

Statistical analysis

Values are expressed in percentages, calculated for each compound and subject, as a division of every original dialysate value over the average of the first eight samples and then multiplied by 100 [29]. The resulting values were been corrected to eliminate the slow continuous changes in dialysate concentrations seen with long periods of dialysis. We calculated the slope of the line for each compound and subject over the entire 30-hr sample period and then corrected the drift. Once the temporal tendency of the data was corrected we analysed, first individually and then populationally, their fit to a sinusoidal model by the Cosinor method, to study the possible circadian variations of values in the three experimental conditions [30]. Where this fit was not statistically significant, but changes in extracellular concentrations between darkness and light were observed, we applied two linear regression analyses between samples 1 and 24 and samples 25 and 48. If the slopes were significant and with different signs we considered this variation as a significant circadian variation.

Results

In the striatum under 12:12 conditions, DA extracellular levels did not follow a sinusoidal model but decreased by 3.26% per hour of basal level (0.75 ± 0.06 nm) in the first transition dark:light (n = 5, r = −0.42, P < 0.001), and increased 1.9% per hour of the basal levels in the second transition light:dark (n = 5, r = 0.30, P = 0.001). DA metabolites, DOPAC and HVA, followed a sinusoidal model (n = 8, P < 0.001; n = 8, P < 0.001). Extracellular levels of 5-HIAA, followed a sinusoidal model too (n = 6, P = 0.053). DA and its metabolites, DOPAC and HVA, as well as 5-HIAA, fluctuated in a circadian rhythm under 12:12 conditions in the striatum (Fig. 2).

Extracellular levels of GLU followed a sinusoidal model (n = 7, P < 0.01), whereas GABA did not fluctuate in a circadian variation although six of seven animals of the group fluctuated in a circadian rhythm following a sinusoidal function when analysed individually. Extracellular concentrations of ARG did not follow a sinusoidal model but decreased by 0.76% per hour during the light period (n = 7, r = −0.14, P < 0.05) and increased by 1.07% per hour during the following dark period (n = 7, r = 0.23, P < 0.01). No circadian variation was found in GLN concentrations. GLU, GABA and ARG, but not GLN, varied in a circadian rhythm under 12:12 conditions in the striatum (Fig. 2).

Under constant light conditions, extracellular levels of DA adopted a sinusoidal pattern (n = 5, P = 0.012). Extracellular levels of DOPAC did not follow a sinusoidal model but decreased by 0.7% per hour of basal level (742.61 ± 96.80 nm) in the transition dark:light (n = 8, r = −0.19, P < 0.01) and increased 1% per hour of basal level throughout the light period (n = 8, r = 0.40, P < 0.001). Extracellular levels of HVA (n = 8) did not fluctuate in a circadian manner although the eight animals studied fitted into a sinusoidal model when analysed individually (P < 0.05). Circadian changes were not found in the extracellular levels of 5-HIAA (n = 8) (Fig. 3). Extracellular concentrations of GLU and GABA followed a sinusoidal model (n = 9, P < 0.01; n = 9, P < 0.01). Neither ARG nor GLN fluctuated in a circadian manner (Fig. 3). Constant light conditions did not disrupt DA, DA metabolites, GLU or GABA circadian rhythms, but did alter the 5-HIAA and ARG circadian rhythms in the striatum.

Under constant dark conditions, extracellular levels of DA (n = 5), DOPAC (n = 7), HVA (n = 7) and 5-HIAA (n = 7) did not vary in a circadian manner in the striatum (Fig. 4). GLU did not follow a sinusoidal model, although four of five animals conforming the group showed a circadian variation of GLU when taken separately. However, the extracellular concentrations of GLU decreased by 4% per hour during the first 12 hr of the experiment (n = 5, r = −0.56, P < 0.001) and increased by 2.4% per hour during the following 12 hr (n = 5, r = 0.5, P < 0.001). Although in all the animals making up the group, the levels of GABA adopted a sinusoidal model, the
populational analysis showed no significant circadian variation. Neither GLN nor ARG concentrations fluctuated in a circadian manner (Fig. 4). Constant dark conditions disrupted DA and its metabolites, and 5-HIAA and ARG, but not the GLU and GABA circadian rhythms in the striatum.

In the nucleus accumbens under 12:12 conditions, DA extracellular levels did not follow a sinusoidal model but decreased by 3.4% per hour of basal level (0.36 ± 0.08 nM) in the first transition dark:light (n = 3, r = −0.42, P < 0.001) and increased 3% per hour of the basal level in the second transition light:dark (n = 3, r = 0.47, P < 0.001). Its metabolites, DOPAC and HVA, followed a sinusoidal model (n = 7, P = 0.014; n = 8, P < 0.01). Extracellular levels of 5-HIAA decreased by 1.14% of basal level (118.92 ± 12.23 nM) in the first transition dark:light (n = 7, r = −0.31, P < 0.001) and increased 0.6% per hour of the basal level in the second transition light:dark

![Fig. 2.](image_url) The striatum dialysate concentrations of DA, DOPAC, HVA, 5-HIAA, GLU, GABA, GLN and ARG as a function of time under control conditions (6 hr darkness:12 hr light:12 hr darkness).

![Fig. 3.](image_url) The striatum dialysate concentrations of DA, DOPAC, HVA, 5-HIAA, GLU, GABA, GLN and ARG as a function of time under light conditions (6 hr darkness:24 hr light).
Fig. 4. The striatum dialysate concentrations of DA, DOPAC, HVA, 5-HIAA, GLU, GABA, GLN and ARG as a function of time under dark conditions (6 hr darkness:24 hr darkness).

Fig. 5. The nucleus accumbens dialysate concentrations of DA, DOPAC, HVA, 5-HIAA, GLU, GABA, GLN and ARG as a function of time under control conditions (6 hr darkness:12 hr light:12 hr darkness).

(n = 7,  r = 0.17,  P < 0.05). DA and its metabolites, DOPAC and HVA, as well as 5-HIAA, varied in a circadian rhythm under 12:12 conditions in the nucleus accumbens (Fig. 5).

Extracellular concentrations of GLU adopted a sinusoidal model (n = 6,  P < 0.05), extracellular concentration of GABA did not fluctuate in a significant circadian rhythm, although five of eight animals followed a sinusoidal model when analysed individually. GLN and ARG did not vary in a circadian rhythm. GLU and GABA, but not ARG and GLU, fluctuated in a circadian rhythm under 12:12 conditions in the nucleus accumbens (Fig. 5).

Under constant light conditions, DA extracellular levels did not adopt a sinusoidal pattern but decreased by 6.14% per hour of basal level (0.37 ± 0.05 nm) in the transitions dark:light (n = 3,  r = −0.47,  P < 0.001) and increased 2.46% per hour of basal levels in the light period (n = 3,  r = 0.51,  P = 0.001), whereas extracellular...
levels of its metabolites, DOPAC and HVA, followed a sinusoidal model ($n = 7$, $P < 0.05$; $n = 7$, $P < 0.01$). Extracellular levels of 5-HIAA ($n = 7$) did not fluctuate in a circadian manner (Fig. 6). Extracellular levels of GLU did follow a sinusoidal model as well as extracellular levels of GABA ($n = 8$, $P < 0.01$; $n = 8$, $P < 0.01$). Dialysate concentrations of GLN and ARG did not vary in a circadian manner (Fig. 6). Constant light conditions did not disrupt the DA and its metabolites, GLU and GABA circadian rhythm, but did alter the 5-HIAA circadian variation.

Dopamine extracellular levels did not fluctuate in a circadian rhythm under constant dark conditions ($n = 6$) although four of six animals studied followed a sinusoidal model individually ($P < 0.05$). Extracellular concentrations of its metabolites, DOPAC and HVA, followed a sinusoidal model ($n = 11$, $P < 0.001$; $n = 11$, $P < 0.001$). The extracellular levels of 5-HIAA also followed a sinusoidal model ($n = 11$, $P < 0.01$) (Fig. 7). Under constant dark conditions, extracellular levels of GLU, as well as the extracellular levels of GABA, followed a sinusoidal model ($n = 10$, $P < 0.001$; $n = 9$, $P < 0.001$). Neither

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**Fig. 6.** The nucleus accumbens dialysate concentrations of DA, DOPAC, HVA, 5-HIAA, GLU, GABA, GLN and ARG as a function of time under light conditions (6 hr darkness:24 hr light).

**Fig. 7.** The nucleus accumbens dialysate concentrations of DA, DOPAC, HVA, 5-HIAA, GLU, GABA, GLN and ARG as a function of time under dark conditions (6 hr darkness:24 hr darkness).
GLN nor ARG fluctuated in a circadian variation (Fig. 7). Constant dark conditions did not disrupt any of the circadian rhythms observed in the nucleus accumbens.

**Discussion**

In these series of experiments we investigated whether simultaneously determined DA, GLU and GABA fluctuated in a circadian rhythm in two different structures of the brain, namely the striatum and nucleus accumbens. The DA metabolites, DOPAC and HVA, and the main serotonin metabolite, 5-HIAA, were also measured. We also investigated whether these rhythms of DA, GLU and GABA in the striatum and nucleus accumbens were modified by light.

In the striatum, and under 12:12 conditions, a circadian rhythm in the extracellular concentrations of DA and GLU was found. DOPAC, HVA and 5-HIAA also fluctuated in a circadian rhythm. No circadian rhythm was found in the extracellular concentrations of GABA, although six of seven animals conforming the group fluctuated in a significant circadian rhythm when analysed individually. Several studies, including one from our own laboratory, have reported the presence of circadian rhythms in the extracellular levels of DA and its metabolites, DOPAC and HVA, serotonin and its metabolite, 5-HIAA, and glutamate with a maximal nocturnal increase [2–4, 17, 31, 32]. These data agree well with the results reported here. In what refers to the extracellular concentrations of GABA, the variability observed may be due to the acrophase variability among individuals as interindividual variations of the circadian rhythm within the same species have been reported [33].

Extracellular concentration of ARG in the striatum significantly decreased with light and increased in the dark. This circadian variation, although it did not follow a sinusoidal function, is consistent with previous results in our laboratory [17]. As ARG is the metabolic precursor of nitric oxide, ARG cyclicity may reflect the activity of the synthesis of nitric oxide in the striatum. In fact, several studies have shown a circadian variation of nitric oxide in other areas of the brain [34, 35]. GLN did not fluctuate in a circadian rhythm in any of the experimental groups studied.

In the nucleus accumbens and under 12:12 conditions, extracellular concentrations of DA and GLU as well as of DOPAC, HVA and 5-HIAA fluctuated in a clear circadian rhythm. In contrast, no circadian rhythm for GABA was observed, despite the fact, as indicated for the striatum, that five of eight animals did fluctuate, individually in a circadian rhythm. As for GABA in the striatum, we assume that GABA varies in a circadian manner in the nucleus accumbens. Neither ARG nor GLN fluctuated in a circadian rhythm in this structure. Shieh et al. [36] reported rhythmic changes in the firing rate of dopaminergic neurones during a 24-h cycle with a nocturnal maximum increase. The circadian rhythm with a nocturnal maximum increase for DA and its metabolites, DOPAC and HVA, in this structure has been previously described [3, 4, 31]. Our results are in agreement with these reports. In contrast, Schade et al. [37] showed a circadian rhythm for DA in the nucleus accumbens although with a diurnal maximum increase. The extracellular concentrations of 5-HIAA fluctuated in a circadian rhythm with nocturnal maximal increases, which is in agreement with previous studies [3, 4]. As far as we know, this is the first study showing the circadian variation of GLU and GABA in the nucleus accumbens.

In the striatum, constant light conditions did not disrupt the circadian rhythm of DA, GLU and DOPAC but they did change the circadian rhythmicity of 5-HIAA and ARG. HVA did not fluctuate in a circadian rhythm but all the animals conforming the group followed a circadian rhythm when analysed individually. We assume that HVA circadian rhythm was not disrupted by light conditions. By contrast, when the rats were kept in constant dark, DA, DOPAC, HVA and ARG (but not GLU) circadian rhythms were disrupted. GABA did not fluctuate in a circadian rhythm, but all animals making up the group fluctuated in a clear circadian rhythm, so we assumed that the GABA circadian rhythm was not disrupted in either light or dark conditions.

The release of DA in the striatum, and also of DOPAC and HVA, fluctuates in a circadian rhythm, which is dependent on the alternation of the light–dark periods. This rhythm was disrupted when the animals were under constant dark conditions. These data would suggest that light is an important determinant of the circadian functioning of DA in this area of the brain. It is possible that the serotonergic system, which has been thought to modulate the response of the suprachiasmatic nucleus to light [16, 38], also plays a role in modulating the responses of DA in the striatum. The fact that 5-HIAA, the metabolite of serotonin, lost its circadian rhythm in dark conditions supports this suggestion. Moreover, the fact that serotonin is released in the striatum through fibres originated in the dorsal raphe [39, 40] and that the terminals of these serotonin are in apposition to the dopaminergic terminals on GABA neurones, and that serotonin exogenously or endogenously applied modulates the release of DA in the striatum, gives further support to this last interpretation [40–43]. At present, it is difficult to explain the maintenance of a circadian rhythm of DA, DOPAC and HVA in light conditions when 5-HIAA does not fluctuate a circadian rhythm. It is possible that in this last experimental situation other systems are implicated in the generation of DA circadian activity. Besides, it is of interest the possibility for melatonin to regulate dopaminergic transmission in the striatum. In fact, Khaldy et al. have shown recently that melatonin decreases dopamine levels in this area of the brain [44]. As, in our experiments, constant darkness disrupts the DA circadian rhythm and as an extension of dark phase prolongs the melatonin peak [45], the possibility for melatonin to play a role in this response is likely.

In the nucleus accumbens and under constant light or dark conditions, DA, GLU and GABA fluctuated in a circadian rhythm, and a circadian rhythm of DOPAC and HVA was found in both conditions. Although, as a group, DA did not seem to fluctuate in a circadian rhythm in dark conditions, a circadian rhythm of this neurotransmitter was found in four of six animals. Moreover, the fact that the dopaminergic metabolites, DOPAC and HVA, fluctuate in a circadian rhythm in the same conditions strongly suggests that DA itself varies in a circadian rhythm [4, 5, 32, 36]. As stated in other parts of this discussion, the lack of
populational statistical significance may be due to a heterogeneity in acrophases and amplitudes between the subjects of the group of study. This amplitude and acrophase heterogeneity (as well as the one observed in GABA) could be due to the different capacity of adaptation to the new light/dark condition of the different animals. Light conditions but not dark conditions disrupted the 5-HIAA circadian rhythm.

In both structures, the striatum and nucleus accumbens, GLU and GABA fluctuate in a circadian rhythm which seems to be independent of light, temperature and food availability, so an endogenous mechanism could be responsible for this neurotransmitter circadian regulation. A similar suggestion has been made for the circadian variations of acetylcholine measured in prefrontal cortex [9].

In summary, this study shows that the neurotransmitters DA, GLU and GABA do have a circadian rhythm in both the striatum and nucleus accumbens. The rhythms in these two structures of the brain are affected differently by changes in environmental light. In the striatum DA, but not GLU and GABA, seems to be influenced by light. In contrast, in the nucleus accumbens these same neurotransmitters have a circadian rhythm independent of light. Further studies will be needed to elucidate which neural pathways, neurotransmitters or hormones are involved in these changes.

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References


