FLUORESCENT LIGHT INDUCES NEURODEGENERATION IN THE RODENT NIGROSTRIATAL SYSTEM BUT NEAR INFRARED LED LIGHT DOES NOT.

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ABSTRACT: We investigated the effects of continuous artificial light exposure on the mouse substantia nigra (SN). A three month exposure of C57Bl/6J mice to white fluorescent light induced a 30% reduction in dopamine (DA) neurons in SN compared to controls, accompanied by a decrease of DA and its metabolites in the striatum. After six months of exposure, neurodegeneration progressed slightly, but the level of DA returned to the basal level, while the metabolites increased with respect to the control. Three month exposure to near infrared LED light (710 nm) did not alter DA neurons in SN, nor did it decrease DA and its metabolites in the striatum. Furthermore mesencephalic cell viability, as tested by [3 H]DA uptake, did not change. Finally, we observed that 710 nm LED light, locally conveyed in the rat SN, could modu late the firing activity of extracellular-recorded DA neurons. These data suggest that light can be detri mental or beneficial to DA neurons in SN, depending on the source and wavelength.

Introduction Light pollution is caused by the excessive and inappropriate introduction of artificial light by humans, directly or indirectly, into the outdoor and indoor environments. Meanwhile, a growing awareness of the adverse impacts of artificial light has led to recog nition of light pollution as a significant global environmental issue (Bennie et al., 2014). Light pollution is considered detrimental to human health due to its effect on temporal organised and properly synchronised internal clocks. Clock synchronisation is caused by circadian light impinging on the retina, which sends a signal to the suprachias matic nucleus in the hypothalamus, leading to a cascade of hor monal changes in the pituitary, pineal and thyroid glands. Nevertheless, light may have other effects attributable to a direct effect on opsins located outside the retina. For example, the sea sonal cycle of reproduction in birds is regulated by opsin 5 (Nakane et al., 2010). Furthermore, light could act on photosensi tive molecules other than opsins. The best known example of photo-activation is the transformation of 7-dehydrocholesterol in Vitamin D3 (Field and Newton-Bishop, 2011). Light pollution exerts several effects on human health but it has never been considered a risk factor for neurodegenerative diseases, in particular, for Parkinson's disease (PD). However, our preclinical and epidemiological studies raised the possibility that prolonged exposure to light might have a role in neurodegenerative mecha nisms leading to PD (Romeo et al., 2013, 2014). We found that 3 months of continuous exposure of rats to bright light, emitted by fluorescent tubes (commonly used in indoor places of work and study), caused a large increase in neuromelanin granules and a significant (29%) reduction of tyrosine hydroxylase (TH)- positive neurons in the substantia nigra (SN). Nigral cell loss was paralleled by decreased tissue levels of dopamine (DA) and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) in the stria tum. The fact that striatal levels of 5-hydroxytryptamine (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) did not change after light exposure indicated that the light-induced neu ronal loss was specific to DA neurons. Furthermore, a loss of TH positive neurons was also found in blind animals subjected to con tinuous bright light exposure, thus suggesting that this loss was due to a direct effect of light reaching the SN rather than an indi rect effect leading to alterations in circadian rhythms (Romeo et al., 2013). Light penetration in biological tissues has been studied for many years owing to its therapeutic and diagnostic potential (Bednarski et al., 2013; Lim, 1989). The study of light penetration

in brain has been done ex vivo in both animals and humans (cada ver heads), and in vivo in experimental animals (for a review see Hamblin, 2016 and reference therein). The first study on light pen etration in human dates back to 1977, when Jöbsis (1977) showed that light photons can travel a 13.3 cm path from side-to-side of the human head. In 10 s, he was able to register, from one side of the head, a higher than background (darkness) photon count emit ted from a light source positioned at the contralateral side of the head. Penetration of light depends on its wavelength and on the com position of irradiated tissues. The lower absorbance, and conse quently, the best transmission of light in tissues, occurs in the red and near infrared range (between 600 and 1300 nm), which is a region known as the ‘‘diagnostic and therapeutic window” (Taroni et al., 2003). In the present study, we initially tested the detrimental effect of fluorescent light on dopaminergic neurons in mice. We compared the detrimental effect of this light on albino vs. pigmented mice, and on young vs. aged mice. Then we recorded the penetration of the fluorescent light across the mouse head in substantia nigra, and we characterized the peak at 710 nm as the most penetrating one. The effect of this wavelength emitted by a 710 nm light emit ting diode (LED) lamp was subsequently tested on mouse dopaminergic cell viability in SN and on mesencephalic cells in cul ture. Finally, we investigated whether brief intranigral application of this 710 nm LED light could modulate the firing pattern of extracellular-recorded neurons in the SN pars compacta.

2. Results 2.1. Fluorescent light-induced reduction of TH-positive neurons in the SN of C57Bl/6J 28 days old mice Initially, we tested whether fluorescent light has detrimental effect on TH-positive neurons in the SN of C57Bl/6J 28 days old mice. Exposure of mice for three and six months to continuous bright light caused a reduction of TH-positive neurons in the SN. The stereological counting of TH-positive neurons in the left SN in animals kept for three and six months under a dim light-dark cycle gave values of 6369 ± 519 and 6001 ± 321, respectively (Fig. 1a and b). In animals maintained in dim light-dark cycle and then exposed to bright light for three months, there was a 32% decrease in TH-positive neurons compared to control animals ($p < 0.05$, Student t-Test), while mice exposed to bright light for six months showed a 40% loss of TH-positive neurons with respect to control animals of the same age ($p < 0.05$, Student t-Test). 2.2. Biochemical assays of neurotransmitters in the striatum of C57Bl/ 6J 28 days old mice treated with fluorescent light The striatal DA content measured in mice kept under dim light dark cycle was 37.3 ± 1.7 ng/mg of protein, while the levels of DOPAC and homovanillic acid (HVA) were 5.20 ± 0.27 and 3.97 ± 0.2 ng/mg of protein, respectively (Fig. 1c). Mice kept under dim light-dark cycle and then exposed to bright light for 3 months showed a significant ($p < 0.05$, Student t-Test) reduction of striatal DA (29.7%) and its metabolites, DOPAC and HVA (24.9% and 29%, respectively) (Fig. 1c). In contrast, in animals continuously exposed to bright light for 6 months, DA returned to control level (Fig. 1d). As far as DA metabolites were concerned, DOPAC almost doubled ($p < 0.05$, Student t-Test) increasing from 3.66 ± 0.38 ng/mg of pro tein in the control to 6.67 ± 0.53 ng/mg of protein in the six months light treated mice, while HVA raised from 3.63 ± 0.26 ng/mg of pro tein in the control to 4.13 ± 0.147 ng/mg of protein in the light treated mice, without reaching levels of significance (Fig. 1d). As an internal control, we measured the levels of noradrenaline (NA), 5-HT and its metabolite 5-HIAA in the same animals in which DA and its metabolites were analysed. The striatal content of NA, 5- HT and 5-HIAA in mice maintained in dim light-dark cycle was 1.91 ± 0.17, 5.24 ± 0.28 and 1.85 ± 0.1 ng/mg of protein, respec tively. In mice treated for three and six months with bright light, the content of 5-HT or 5-HIAA did not change while the content of NA increased at three months to 2.89 ± 0.18 and returned at basal level of 1.8 ± 0.14 after six months (Fig. 1c and d). 2.3. Stereological counting and neurotransmitters assays in albino C57Bl/6J/Tyr mice exposed to fluorescent light We then decided to test whether the same detrimental effect seen in black mice could be reproduced in albino mice. The stere ological counting of TH-positive neurons in the SN of albino

C57Bl/6J/Tyr mice kept under dim light-dark cycle gave a mean value of 6253 ± 388 (Fig. 2a), while the DA content was 31.8 ± 2.16 ng/mg of protein (Fig. 2b). The levels of DA metabolites DOPAC and HVA were 9.01 ± 1.25 and 4.7 ± 0.47 ng/mg of protein, respectively. Albino C57Bl/6J/Tyr mice kept under dim light-dark cycle and then exposed to bright light for 3 months showed a 31% reduction of TH-positive neurons ($p < 0.05$, Student t-Test) (Fig. 2a), while the DA content was 32 ± 2.1 ng/mg of protein, which was not signifi cantly lower compared to control (Fig. 2b). In contrast to DA, its metabolites DOPAC and HVA were significantly ($p < 0.05$, Student t-Test) reduced by 35.4% and 23.4%, respectively compared to con trol (Fig. 2b). The striatal contents of 5-HT and 5-HIAA were not significantly altered after bright light exposure compared to con trol mice, while the content of NA increases significantly from 1.05 ± 0.15 to 1.77 ± 0.12 (Fig. 2b). 2.4. Stereological counting and neurotransmitters assays in C57Bl/6J one year old mice exposed to fluorescent light In this set of experiments we tested the detrimental effect of fluorescent light on aged mice. The stereological counting of TH positive neurons in the SN of one year old mice maintained under dim light-dark cycle gave a value of 5840 ± 379 (Fig. 3a), while the DA content was 30.7 ± 1.47 ng/mg of protein (Fig. 3b). The DOPAC and HVA levels were 4.45 ± 0.4 and 3.06 ± 0.21 ng/mg of protein, respectively. One year old mice grown under dim light-dark cycle and then exposed to bright light for 3 months showed a not signif icant 15% reduction of TH-positive neurons compared to the con trol. Although DA content did not significantly change compared to the control, the DOPAC and HVA level, were significantly reduced by 25.8% and 17%, respectively ($p < 0.05$, Student t-Test) (Fig. 3b). The striatal content of NA, 5-HT and 5-HIAA did not change after bright light exposure as compared to control mice (Fig. 3b). 2.5. Assay of urinary corticosterone after fluorescent light exposure We measured urinary corticosterone levels to evaluate whether the detrimental effect of fluorescent light observed in mice SN could be due to a stress-induced change in circadian rhythm. This method was chosen because it is less invasive and results in less handling-associated stress than collecting serum and faeces. Fur thermore, corticosterone is not subject to microbial metabolism in urine as would occur in faeces. We found that after fluorescent light exposure, corticosterone levels greatly increase at 24 h, rising from 14.15 ± 6.98 to 49.94 ± 8.98 before and after light exposure, respectively. Values of urinary corticosterone returned to basal levels already after 48 h, despite continued fluorescent light expo sure (Fig. 4). Control mice maintained under dim light-dark cycle, instead, did not show any alteration in urinary corticosterone levels throughout the experimental period. 2.6. Penetration of light in the mouse SN In order to see which component of the florescent light was able to penetrate across the mouse head and reach the SN, by means of an optical probe stereotaxically inserted in the SN, we measured the light spectrum originating from a fluorescent lamp, positioned 20 cm in front and above the mice head. For this measurement, we used a small lamp with a light flux that was about 9-fold lower respect to that used for the whole animal exposure (Fig. 5a). We were able to measure in SN only wavelengths above 600 nm with peaks at 610 and 710 nm. The amount of light reaching the SN of the albino CD1 mice at 610 and mainly at 710 nm (Fig. 5b) was higher than that measured in C57Bl/6J black mice (Fig. 5c). By covering the eyes with aluminium foil, light penetration was strongly reduced in albino CD1 mice (Fig. 5b), while only slightly in C57Bl/6J black mice (Fig. 5c). The fluorescent lamp we used to measure light penetration in SN, has a tiny peak at 810 nm (Fig. 5a). It is very likely that the penetration of this wavelength in SN is better than those at 610 and 710 nm, nevertheless, we did not consider this wavelength, because its signal in SN is indistinguishable from the background noise (Fig. 5b and 5c). 2.7. Stereological counting and neurotransmitters assays in C57Bl/6J mice exposed to 710 nm LED light Given the above results, we tested whether light at 710 nm was detrimental to DA neurons in SN, to this end we exposed C57Bl/6J mice to a 710 nm LED lamp for three months. The stereological counting of TH-positive neurons in the total extended SN (right and left sides) of C57Bl/6J mice kept under dim light-dark cycle gave a mean value of 10,140 ± 389.62

(Fig. 6a), while the DA con tent was 49.9 ± 3.11 ng/mg of protein (Fig. 6b). The levels of DA metabolites DOPAC and HVA were 8.61 ± 0.94 and 6.36 ± 0.47 ng/ mg of protein, respectively. C57Bl/6J mice kept under dim light dark cycle and then exposed to 710 nm LED light for 3 months showed no reduction of TH-positive neurons in SN (mean value of 11572.8 ± 822.72) (Fig. 6a), and no variation of DA or metabolite content was seen in the striatum (Fig. 6b) with respect to controls. Furthermore, the striatal contents of NA, 5-HT and 5-HIAA were t significantly altered after LED light exposure compared to con trol mice (Fig. 6b). 2.8. Effect of 710 nm LED light exposure on [3 H]DA uptake and DA content in mesencephalic cell cultures from 13 day-old C57Bl/6J mice embryos The effect of light at 710 nm was also tested on mesencephalic cell cultures. They were exposed for 20 min for either one or four days to a 710 nm LED lamp, then dopaminergic cell functioning was assayed by evaluating the [3 H]DA uptake and the DA content. As shown in Fig. 7, in light-treated cells, the [3 H]DA uptake did not change compared to the control. In contrast, DA content was signif icantly decreased after both one and four days of light exposure ($p < 0.05$). 2.9. C57Bl/6J mice electroretinographic (ERG) responses to white, blue (460 nm) and red (710 nm) LED light Even though corticosterone levels did not show any stress induced change in circadian rhythm, we further explored whether there was a difference in the stimulation of the retina between the different light sources. To this aim, we compared the electroretinographic responses of C57Bl/6J mice stimulated by different wave length LED light sources. Flash-ERG responses are characterised by a negative wave (a-wave) followed by a positive wave (b wave). The amplitude of these waves is correlated respectively to photoreceptor activation and to the transferral of visual informa tion from photoreceptors to second order neurons in the retina. In the electroretinogram, we measured the amplitude of the b wave. In mice stimulated with white light at 0.01 cd/m2 the b wave amplitude was 213.83 mV (Fig. 8a). Monochromatic, blue (460 nm) and near infrared (710 nm) light at around 100 lW/cm2 - nm, gave b-wave of 116.68 mV and 125.75 mV amplitude, respec tively (Fig. 8b and c). 2.10. Effects of light stimulation on the discharge activity of neurons in the pars compacta of the rat SN Finally, as infrared light have been shown to be neuroprotective rather than detrimental for dopaminergic neurons in substantia nigra (see Discussion for references), we tested whether it has any effect on neuronal firing. Light stimulation was tested on 39 neurons recorded in the pars compacta of the SN. It is important to note that the peak of the 710 nm LED light source, used to stim ulate the presumed dopaminergic neurons, included in this region, moved from 710 to 720 nm, when powered at 1.98V and 80 mA (Fig. 13c). The recorded neurons showed a low frequency and slight irregular discharge (2–6 Hz) (Fig. 9a), and the wide triphasic impulse with the initial segment-somatodendritic break in the ascending phase of the impulse wave, typical of dopaminergic neu rons (Berretta et al., 2010; Grace and Bunney, 1983, 1984) (Fig. 9a). Of the 39 neurons, 30 were responsive to light stimulation (77%) (Table 2). The response was typically an increase of firing which started after a latency of 60.0 ± 20.0 s. The frequency discharge of the 30 neurons before stimulation was 2.2 ± 0.4 imp/s. The dis charge pattern was slightly irregular, as shown by the positively skewed inter spike intervals (ISI)s distribution (Fig. 9b). The firing significantly increased to 8.8 ± 1.9 imp/s during the stimulation period ($p < 0.05$) (Table 2). When stimulation ceased, firing did not significantly decrease (7.2 ± 2.3 imp/s) compared to the stimu lation period, but maintained significantly higher values compared to the pre-stimulus period ($p < 0.05$) (Fig. 9c). We occasionally observed, during the activation of an individual neuron, that one or two previously silent neurons could be activated above electri cal background noise. These neurons were not detected by the pre fixed on line threshold discriminator procedure that was set to select the higher amplitude of the spontaneously active neuron, thus this was not included in the total number of recorded neu rons. In addition to the firing increase induced by stimulation, the responsive neurons showed a net change in the discharge pat tern. This was due to the occurrence of bursts of 3–5 spikes, which reflected the shorter mean ISI observed during stimulation (Fig. 9b). The values of skewness and kurtosis during stimulation

were significantly higher with respect to the pre-stimulation per iod ($P < 0.05$) (Table 2), thus causing an increased positively skewed distribution of ISIs. The modes, skewness and kurtosis showed that neurons switched from a slight irregular discharge to a burst-discharging pattern. A reconstruction of recording track, histological evidence of the recorded site and position of respon sive and not responsive neurons to light stimulation is reported in Fig. 10a, b and c, respectively. In control experiments, we analysed the activity of neurons in the ventrobasal thalamic nuclei. Neither the firing rate nor the dis charging pattern of the 7 neurons recorded in this area was shown to be affected by the 4 min of light administration (Fig. 11and Table 3). A reconstruction of recording track, histological evidence of the recorded site and position of not responsive neurons to light stimulation is reported in Fig. 12a, b and c, respectively.

. Discussion In this study, we reported that the detrimental effect of contin uous fluorescent light exposure, previously observed in rats (Romeo et al., 2013), also occurs in the DAergic neurons of the mouse SN. A three month continuous exposure of C57Bl/6J mice to fluores cent light induced a reduction of about 30% in DA neurons in SN compared to controls, accompanied by a quantitatively compara ble decrease loss of DA and its metabolites DOPAC and HVA in the striatum. After six months of exposure, neurodegeneration pro gressed slightly, but, surprisingly, the level of DA returned to the basal level, while the two metabolites increased with DOPAC dou bling respect to control. These results suggest that when DA neu rons are reduced below a critical number, the remaining neurons may compensate the loss by establishing new synapses and syn thesising a larger amount of DA, which results in an increase in metabolism. Such an effect has been demonstrated by Stanic et al. (2003); who clearly showed that after 16 weeks of a 6- hydroxydopamine-induced partial lesion of DA neurons, axons in the pars compacta of the rat SN establish new synapses and re innervate the dorsal striatum, the DA content returned to normal and DOPAC increased. As an internal control, we also tested the striatal content of NA and 5-HT. Remarkably, while 5-HT and its metabolite 5-HIAA were not altered after three and six months of fluorescent light expo sure, NA increased after three months, and returned to the basal level after six months. We have no elements to interpret this result, but it seems to be a compensatory mechanism for the DA loss. Since C57Bl/6J mice have a black mantle, absorbing and possi bly reducing the amount of light reaching the SN, we performethe same experiments using albino C57Bl/6J/ Tyr white mice mutant for the tyrosinase gene. As predictable, in albino C57Bl/6J/ Tyr mice, the amount of light penetrating the skull and scalp, and reaching the SN was higher than that observed in black mice. Despite this difference, albino mice exposed to a fluo rescent lamp for three months showed a loss of DA neurons in the SN equivalent to that occurring in C57Bl/6J black mice, indicating that the greater light intensity reaching the SN in albino mice with respect to the black mice was not enough to cause increased dam age or, as indicated above, there was a threshold beyond which compensatory mechanisms prevented further damage. Surpris ingly, DA was not altered in these albino mice while the metabo lites DOPAC and HVA were reduced. As shown above for C57Bl/6J mice, 5HT and its metabolite were not altered, while NA was clearly increased. Since it has been shown that aged mice are more sensitive than young adults to the neurotoxic effects of 1-methyl-4-phenyl-1,2, 3,6-tetrahydropyridine (MPTP) (Gupta et al., 1986), in an addi tional experiment we tested whether C57Bl/6J one year-old mice were more sensitive to light-induced neurodegeneration. Notwith standing this premise, the reduction of DA neurons in these mice did not reach a level of significance; furthermore, DA was noaltered while its metabolites DOPAC and HVA were significantly reduced. These results indicate that the mechanisms which make aged mice more susceptible to MPTP do not work in the same way when light is used to lesion DA neurons in SN. As shown in the Results, and similar to what has been seen in rats (Romeo et al., 2014), some components of the light emitted by the fluorescent lamp can penetrate through the scalp and the skull and reach the SN in mice. Only wavelengths above 600 nm

were detectable by our radiometer in the SN, with maximal trans mission peaks at 610 and 710 nm in both albino and black mice. The light at 710 nm belongs to the near infrared spectrum, and near infrared light (670 and 810 nm) has been shown to protect DA neurons in substantia nigra from MPTP lesions (Johnstone et al., 2014; Moro et al., 2013; Oueslati et al., 2015; Peoples et al., 2012; Purushothuman et al., 2013; Reinhart et al., 2016a, 2016b, 2015; Shaw et al., 2010). Nevertheless, the neuroprotective effect has been shown for short period of 670 and 810 nm light exposure, then we speculated that continuous exposure to 710 nm light could have detrimental effects on DA neurons in SN. A three month continuous exposure of C57Bl/6J mice to a 710 nm LED lamp did not alter either the number of DAergic neu rons in SN or the level of the neurotransmitter DA, NA and 5-HT in the striatum. It is worth noting that the intensity of the light emit ted by the LED lamp we used to expose the mice, was 4-fold higher than the peak at 710 nm of the fluorescent lamp, than the lack of effect was not due to a reduced power intensity. We expanded our observation by testing whether the 710 nm LED light may directly influence the dopaminergic neurons. The results showed that short periods of exposure of mesencephalic cell cultures to this wavelength slightly but significantly reduced DA content, while did not affect DA uptake. As DA uptake is as an index of DAergic cell viability (Vaglini et al., 1994), the lack of an effect of the LED lamp strengthen the suggestion that this wave length is not detrimental to dopaminergic neurons. Conversely, the reduction in DA content indicates that the wavelength of 710 nm influences the release of DA by mesencephalic dopaminergic neurons. A major issue arising from the above reported results on mes enchephalic dopaminergic cells concerned the possibility that the 710 nm LED light could also induce electrophysiological changes in the activity of dopaminergic neurons of the SN. Since our exper iments were directed to investigate the effects of transcranial exposure to light, we investigated the short-term effects of local opto-stimulation on the firing pattern of presumed DA neuronsin the pars compacta of the SN. We performed the electrophysiolog ical experiments administering light in tight proximity (500– 600 mm) of the dorsal part of the SN, via a stereotaxically inserted thin optical fibre. On the basis of their electrophysiological characteristics, the recorded neurons were likely to be dopaminergic in nature. The major effect of light stimulation was a four-fold increase of sponta neous firing rate and a shifting of firing from a slight irregular pat tern to a burst pattern. Most of the recorded neurons showed a significant increase of their firing rate within 60 s of stimulation, which outlasted the stimulation period. Such short-term changes might represent the beginning of a wider and more sustained effect that might take place under chronic light exposure. In some exper iments, previously silent neurons were also observed to be acti vated in addition to the spontaneously active neuron under investigation, thus suggesting that the action of light could involve a pool of neurons, rather than a single one. Assuming that all of the light has been absorbed in the tissue near the optical probe, we can definitely exclude heat having trig gered the observed changes, since the temperature increase would have been less than 0.1 C in 1 h, as we calculated. Furthermore, this is also indicated by the lack of activity in ventrobasal thalamic nuclei. The detrimental effect of white fluorescent light, in contrast to near infrared light, raises the issue of how white light can damage dopaminergic neurons. One possibility is by altering the circadian rhythm and causing stress to mice. To address this issue, we fol lowed the urinary corticosterone levels in animals exposed to flu orescent light for up to one month. Remarkably, after an early increase after 24 h of continuous light exposure, corticosterone returned to basal levels and remained unaltered for the entire observation period. Our results are in accordance with a recent work by Alves-Simoes et al. (2016), where it is shown that the var ious light environments to which mice were exposed, among them constant light, did not affect the animals' growth rates or stress levels, measured with corticosterone level. This result weakens the possibility that stress induced by alterations of circadian rhythms could be responsible for the observed effect of fluorescent light in SN. In addition, there are two more arguments that point against stress-induced neurodegenerative of DA neurons in SN: 1) in

the previous work (Romeo et al., 2013), we have clearly shown that fluorescent light has the same detrimental effect in SN in rats with a bilateral optic nerve excision as in normal rats; and 2) LED light at 710 nm is able to stimulate photoreceptors in the retina, and potentially to exert changes in circadian rhythm by modulating the activity of melanopsin retinal ganglion cells, but it has no detri mental effect on mice. The wavelength of the fluorescent light that causes DA neuron degeneration remains to be identified. Our data not only exclude that light at 710 nm is not detrimental when it reaches the sub stantia nigra, but they also exclude any indirect effect of this wave length on other targets (Johnstone et al., 2014). Furthermore, since there is not photo-biological difference between light at 710 and 610 nm, it is unlikely that this last wavelength could be responsi ble for the neurodegenerative effect of the fluorescent light. Thus, we have to assume that shorter wavelength (blue/green) are responsible for the detrimental effect of fluorescent light. Since shorter wavelengths penetrate less, it could be that the tiny amount of light reaching the SN is enough to cause neurodegener ation, or in alternative these shorter wavelengths could cause neu rodegeneration by modifying circulating molecules that secondarily reach DA neurons in SN. This last effect is call abscopaeffect, and it was shown by Johnstone et al. for the neuroprotective effect of 670-nm light against the neurodegenerative effect of MPTP (Johnstone et al., 2014). The light mechanism of protection mostly investigated is the mitochondrial increase of electron transfer in the respiratory chain, this effect follows the activation of photo-acceptors such as the cytochrome C oxidase, that in turn results in ATP production and inhibition of apoptosis. Nevertheless, recently are emerging other biological effects of light, like the production of the neuromediator nitric oxide or the alteration in intracellular calcium, that could be responsible for the deleterious effect we have seen (see Hamblin, 2016 and reference therein). In conclusion, regardless of the intimate mechanism by which light acts on dopaminergic neurons, the neuronal loss, the change of the DA content in mesencephalic cell cultures and electrophys iological data provided in this paper altogether offer a physiologi cal starting point from which further studies may be carried out in an attempt to explain the neurodegenerative effect of light in SN neurons following long-term light exposure.