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# Progesterone Prevents Impairment of Cognitive, Affective and Motor Events in a Reserpine Model of Parkinsonism in Male Rats

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## Abstract

Parkinson's disease is a common neurodegenerative disorder, second only to Alzheimer's disease. It has been the object of growing interest since its frequency tends to augment as life expectancy has increased globally considered. In this work, we study the eventual neuroprotective effect of Progesterone ($P_4$) injected a few days before establishing a Reserpine (R) model of Parkinsonism in adult male rats. We focused on cognitive (Novel Object Recognition test; NOR), affective (Forced Swimming Task; FST) and motor (Catalepsy Test; CT) outputs. We performed the appropriate tests along with the administration for two weeks of low doses of R. Our results show very clearly that $P_4$ prevents the impairment of several tasks regarding the deleterious effects of R, without affecting negatively other areas of the subject behavior. In total accordance with current information, we show here that $P_4$ -in particular- and neuroactive steroids -in general- are promising molecules to delay diverse manifestations of neurotransmitters depletion.

**Keywords: Progesterone; Neuroactive steroids; Parkinsonism; Neuroprotection; Male rats**

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## Introduction

Parkinson's disease is a threatening health problem in the world. It is the second most common neurodegenerative disease [1]. Besides many advances in the management of the illness, it remains complicated [2]. This is so -at least in part- because what was once the hallmark of the disease, the loss of dopaminergic neurons in the substantia nigra, has since changed to consider the disease as a multi-systemic degenerative process involving several pathways, structures and neurotransmitters [3]. Ongoing research is highly interested in the possibility of using neuroprotective drugs to avoid the damages of the disease or at least delay them long enough to give the patient a better quality of life.

Neuroactive steroids are a well-known set of molecules that comprise steroids from any source that show effects on the Central Nervous System (CNS). Among them, there are a group of neuroactive steroids-collectively named neurosteroids- that are synthesized the novo in the CNS, and that exert autocrine and paracrine effects only [4]. Both groups of molecules have a renowned target as putative molecules regarding neuroprotection. Progesterone ($P_4$) is one of the major players among them [5]. Parkinson's disease remains elusive in terms of treatment, being one major reason that the disorder, once thought as merely a motor illness, is a complex set of signs and symptoms that involve cognitive, affective and motor expressions [2]. Today, a major goal is to recognize early subtle behavioral (cognitive, affective and motor) signs of malfunctioning to promptly begin with the treatment.

There are several animal models of Parkinsonism that have proved to be very useful in researching several essential aspects of the disease, from behavior to molecules [3]. In this work, we try to study whether or not $P_4$ could be eventually neuroprotective when administered just a few days before of injecting our subjects with several low doses of Reserpine (R) [6]. We selected R as our model since it affects not only dopaminergic neurons but also catecholamines and indoleamine as a whole [3]. What was once a disadvantageous situation, today it has been reverted since the human disease involves a much more complex landscape that the mere loss of dopaminergic

neurons. Additionally, the method-comparatively- is a rapid way of establishing the deficits in the subjects and also testing the effects of putative protective molecules.

## Methods

### Subjects

Male Sprague-Dawley rats, 60 days old, and 450 g average weight were used. Animals were housed in groups of three individuals per cage. Room temperature was maintained at 22°C ± 2°C, on a 12 h artificial light/dark cycle (light from 7:00 am to 7 pm). Commercial rodent food and water were provided ad libitum. All procedures were approved by the local committee for care and use of animals in medical research (CICUAL, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, cicual@fcm.uncu.edu.ar), and experiments were performed in accord with the National Institutes of Health guide for the care and use of laboratory animals.

### Reagents and solutions

Reserpine (R) and Progesterone ($P_4$) were purchased from Sigma Aldrich St Louis MO, USA. R was diluted in glacial acetic acid (final concentration 0.5% acetic acid in distilled water) and subcutaneously administered (SC) at a dose of 0.1 mg/kg. $P_4$ was dissolved in ether, benzyl alcohol was added, and finally, it was resuspended in corn oil. $P_4$ administration was also SC, at a dose of 4 mg/kg.

### Experimental design

The experimental protocol was carried out along 21 days, according to Figure 1. From day -5 to -1 animal received either a daily Intraperitoneal (IP) injection of sterile saline or an SC injection of $P_4$. On odd days from 1 to 13 animals received either daily reserpine or sterile saline according to the group (Figure 1). Catalepsy test was carried out on even days from 2 to 14. Novel Object Recognition test (NOR test) was performed on day 8, and Forced Swimming Test (FST) was performed on day 9.

At the beginning (Figure 1), animals were randomly assigned to one of 4 groups (n=10 each) injected with: 1) Sham (S), saline-saline; 2) saline-reserpine (R); 3) Progesterone-Reserpine (P+R); and 4) Progesterone-Saline (P+S).

### Reserpine model

Reserpine (R), a monoamine depleting agent, is an ester alkaloid derived from *Rauwolfia* sp. root utilized initially for the treatment of hypertension [7]. R was underused for years as a useful reagent to induce animal Parkinsonism since it was not selective for dopamine, the hallmark neurotransmitter whose lack by death of dopaminergic neurons in the substantial nigra was always considered to be the responsible for the pathophysiology of the illness, according to Jenner in 1989 [8]. Today this is not the case anymore as different monoamines besides dopamine (noradrenaline, serotonin) and different nuclei besides the substantia nigra (raphe nuclei and locus coeruleus) are all known to be affected by the disease [3]. Today, R is a well-established model to induce animal Parkinsonism [6].

### Catalepsy test

In the catalepsy test, the subject is placed in an unusual posture and then the observer measures the time that takes to the animal to correct this unusual posture [9]. We used a horizontal Plexiglass bar held between two vertical support bars located at 9 cm from a smooth surface. Animal's front limbs were positioned on the bar and the latency time to step down from the bar was recorded. Three trials were made per rat on each observation. Result for each observation was the mean value. We establish catalepsy like behavior criteria, considered as immobility posture time. Maximum time allowed for the whole test was 180 sec.

### Novel object recognition

The experiment was realized as described elsewhere [10,11]. Briefly, the apparatus consisted of a wooden box (70 cm × 45 cm × 30 cm) with a white acrylic floor. It was located in an isolated testing room that was dimly lit by constant indirect illumination from the primary source, a 25 W light bulb suspended over the box. The objects utilized as familiar (previously experienced object) or unfamiliar (object not previously experienced, i.e. the novel one) were three copies of a pink truncated pyramid and a grayish-opaque candlestick of approximately the same size, all of them heavy enough to prevent accidental displacement by the subjects.

The animals were allowed to get used to the experimental room for at least 1 h. The day before training, each animal freely explored the apparatus with no objects for 2 min. A training session (T1) was followed by a test session (T2) 24 h later. During the training session, animals were placed in the arena containing two identical objects (pink truncated pyramids). In the test session, a familiar object was changed for an unfamiliar one (grayish opaque candlestick). Both training and test stages were 3 min each. The position of the objects (familiar and unfamiliar) and the extreme of the box used to place the objects were randomly exchanged for each experimental animal to avoid the use of potential confounding spatial clues. Exploration was defined as the orientation of the animal's snout toward the object within a range of 2 cm or less from the object. Running around the object or sitting on it was not recorded as exploration. The objects and floor were carefully cleaned with ethanol (10%) after each trial to equate olfactory cues. The experiments were recorded with a camcorder digital camera JVC Everio GZ-MG330 (Japan) using a black and white recording mode to improve the register. The measures in the object recognition test were as follows: 1) total time spent by the subject exploring both objects during training (T1); 2) total time spent by the subject exploring just the novel object during T2; and 3) discrimination index, the difference between time spent exploring unfamiliar and familiar objects during T2.

### Forced swimming test

The FST was slightly modified from the original one [12]. The device consists of a plexiglass cylinder 38 cm in diameter and 60 cm in height. The cylinder contained water at 25°C, 45 cm in height. Rats were individually introduced into the cylinder for 5 min and removed to be dried before returning them to their home cages. The test was videotaped (see above). The following parameters were scored: 1) Immobility time: time spent with the head above the water surface, and the necessary movement to maintained it; 2) swimming time: movements through the apparatus with front limbs inside the water; 3) climbing time: Movements with the front limbs against the cylinder wall; and 4) diving: Events of exploratory movements under the water with all the body submerged.

### Statistical assays

All data were analyzed by a one-way ANOVA test followed by a Tukey post hoc test and expressed as the mean ± SEM. A value of $p<0.05$ was considered statistically significant.

## Results

In the present work, when compared to another study [6], relatively small but repeated doses of R (0.1 mg/Kg) were used to

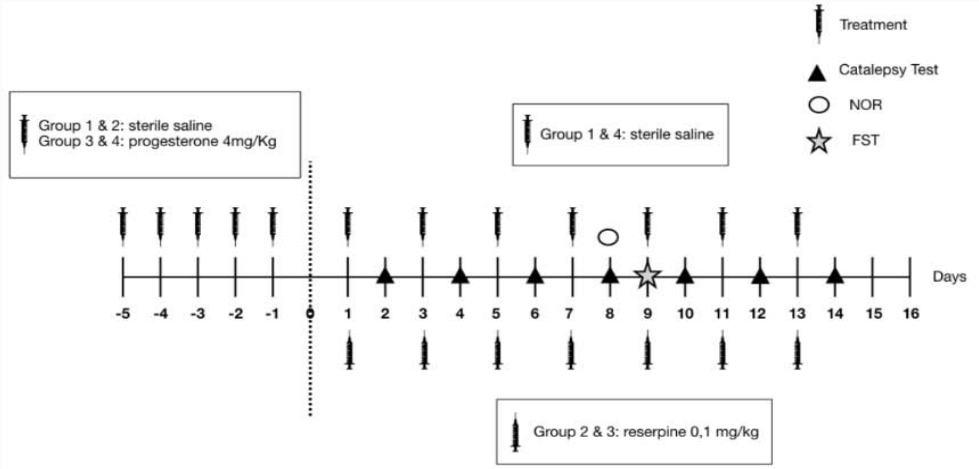


**Figure 1:** Experimental protocol.

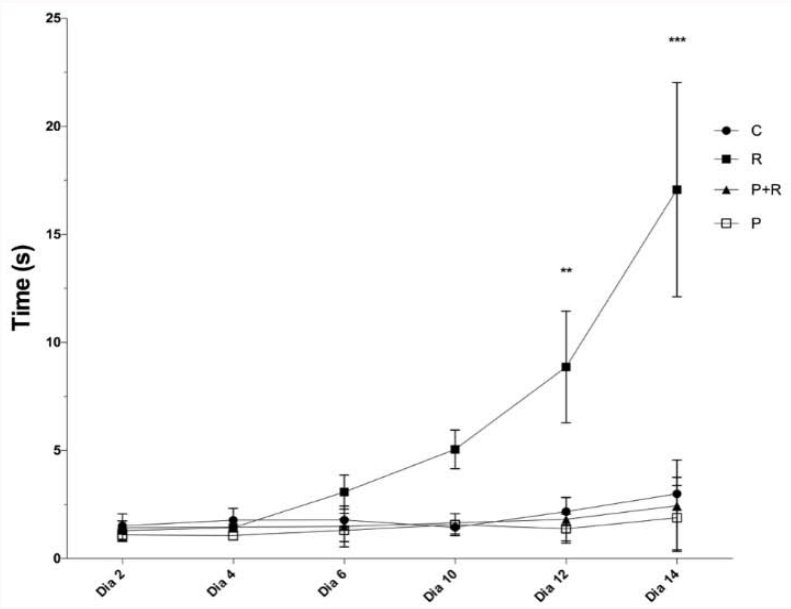


**Figure 2:** Catalepsy latency time.
C = Control group; R = Reserpine group; P = $P_4$ group; P+R = $P_4$ plus reserpine group. Results are expressed as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001.

obtain subtle but reliable motor changes as well as cognitive and affective ones. In this sense, we were able to induce Parkinsonism signs in our subjects since all of the animals treated with R developed changes in each of the testing conditions.

These changes included: a) More time spent in the bar during the catalepsy test, indicating a definite impact on motility, particularly from day 10 of R treatment until reaching a peak on day 14 (Figure 2); b) subjects significantly spent less time swimming during the forced swimming task, which is a clear indication of what is known as a "depression" like behavior in rodent models when compared to controls without treatment or subjects treated with $P_4$ alone (Figure 3). The diving activity was measured by the number of accumulated events. Control group made 21 dives, while in the reserpine group we observe only 4 events.

Reserpine group previously treated with progesterone obtained a cumulative result of 11 dives, while for the group treated only with progesterone the cumulative result was 16 dives; c) also in the recognition of the novel object during the test, controls, and $P_4$ alone treated subjects did not have any problem at all in recognizing the new object. On the other hand, R treated subjects, nonetheless spending the same total amount of time in exploratory behavior -also a clear indicator of what is supposed to be expected from animals that did not suffer from any lack of motivation and/or translation problems (Figure 4) ignored the condition of new object during the test day, indicating an impact on memory, a cognitive component of the model (Figure 5). These results, when taken together, allow us to consider our subjects as a valid model of monoamine depletion. By extension also as a model of premotor and motor signs of Parkinsonism, suitable for being used to study eventual neuroprotection by the neuroactive steroid $P_4$.

## Discussion

Regarding $P_4$, it was striking the effect when administered for five days before the injections of R. In fact, in every condition tested, $P_4$ prevented the impairment of all the components previously

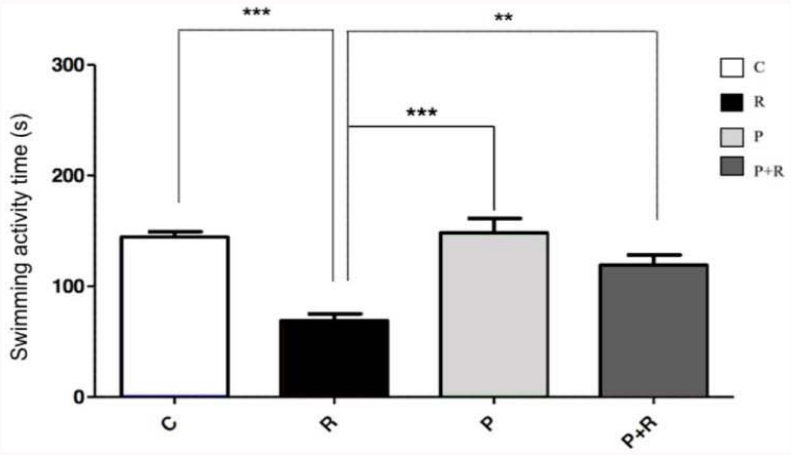


**Figure 3:** Swimming activity time.
C = Control group; R = Reserpine group; P = $P_4$ group; P+R = $P_4$ plus reserpine group. Results are expressed as mean ± SEM. Swimming time in seconds. $^{**}p < 0.01$, $^{***}p < 0.001$.

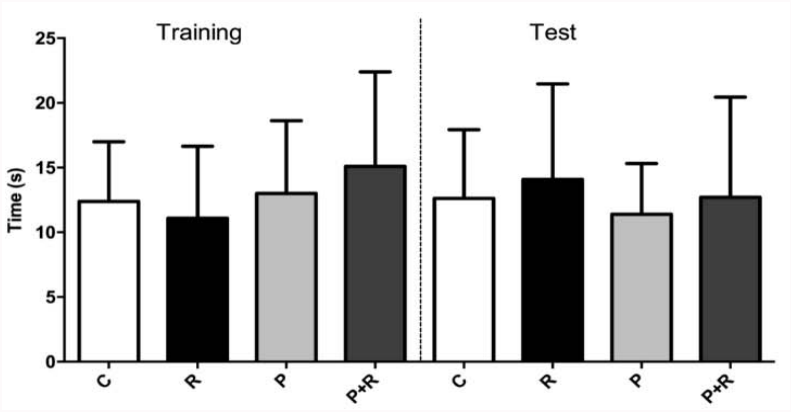


**Figure 4:** Novel object recognition test. Total exploratory time during training and test sessions.
C = Control group; R = Reserpine group; P = $P_4$ group; P+R = $P_4$ plus reserpine group. Results are presented as the mean + SEM expressed in seconds.

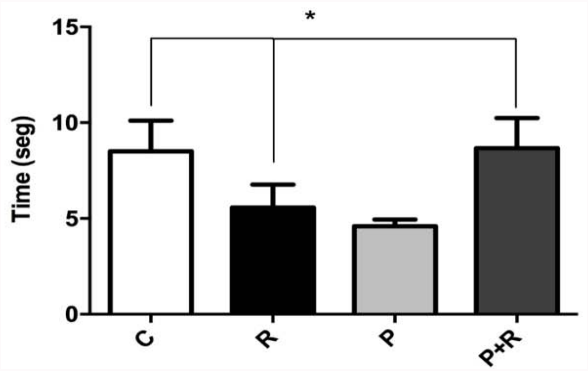


**Figure 5:** Novel object recognition test. Discrimination index.
C = Control group; R = Reserpine group; P = $P_4$ group; P+R = $P_4$ plus reserpine group. The results represent the mean ± SEM expressed as discriminations index (DI) in seconds. $^{*}p < 0.05$.

described, resulting in motor, cognitive and affective performances quite comparable to control subjects. It is important to note that $P_4$ had not any effect per se. Additionally, we must stress here that the model we used in the present work allows us to study the acute effect of neuroprotection instead of addressing neurorestoration, being more useful for the later models based on intrastriatal administration of toxins like 6-Hydroxydopamine (6-OHDA) [13].

Several papers stress the importance of sex steroids and neurosteroids as neuroprotective factors [14,15]. Men are more susceptible than women to Parkinson's disease, at least until women reach menopause [15], which is highly suggestive for a role of sex steroids like estrogen [16]. Moreover, steroid hormones synthesized by the brain have been shown to display neuroprotective properties eventually useful for the prevention and treatment of

neurodegenerative diseases [14]. Probably, what it is particularly interesting here are twofold: 1) the validity of reserpine as a reagent suitable for generating a deficit constellation in rodents that remembers what happens -at least in part- in Parkinson's disease. It does so relatively quickly when compared to another method [3], and it does consistently every time according to our results. Additionally, the whole picture is reversible when R is discontinued; and 2) $P_4$ prevented impairments in all areas-again consistently- when administered for a short time before R administration. There are advantageous animal models of Parkinsonism that are widely used [3]. However, all of them involved some cumulative changes and cell death with compensatory changes as well. In this work, we show short bursts of $P_4$ before R treatment, and a short R treatment to induce the reported malfunctions.

Parkinson's disease is a very complex disorder in which probably many factors contribute to the onset and later stages of the disease [17]. At present, there is no single successful treatment to stop the progression of the illness [17]. As such it results interesting the possibility of addressing the role of several treatments at once acting synergistically to prevent neuronal death or restore brain damage. In this sense -among others- gonadal steroid hormones have been shown to result promising. As we mentioned earlier, since women are less prone than men to present Parkinson's disease [15] at least until menopause- it looks promising to think about gonadal steroids as putative candidates to be one of the combined strategies to treat the disease. In fact, and besides that estrogens have been less contradictory that progestin's in showing beneficial effects, $P_4$ also has been reported to be useful [17]. In our lab, treatment with $P_4$ for 3 days after 6-OHDA injections improved behavioral targets as well as normalized glutamatergic and dopaminergic activities [11,18]. However, here we are dealing with acute effects, instead of the chronic model of neuronal death by 6-OHDA, which poses the question of that, apparently, at least, we could talk of dysfunctional neurons here more than dead cells. Since one of the possible targets of Parkinson's disease is neurotrophic factors [13], it would be possible that $P_4$ before administration of reserpine could -acting quickly- exert their protective effect on membrane progesterone receptors, stimulating neurotrophic factors, such as BDNF. In our lab we have seen an increase in BDNF levels in response to administration of allopregnanolone, an endogenous neurosteroid derived from progesterone that acts as a GABAA positive allosteric modulator (unpublished data). Since nigral Dopamine (DA) neurons are sensitive to several neurotrophic factors (BDNF among them) it would not be impossible to think of $P_4$ as inducing an increase of such neurotrophic factors and then, preventing DA neurons of becoming dysfunctional and hence, avoiding the potential proinflammatory damage of reserpine. Interestingly, Jarras et al. [19] reported that in mice gut -gastrointestinal symptoms are also common in Parkinson's disease- lesioned with 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP) they showed neuroprotection and immunomodulation of DA neurons (they had previously shown also neuroprotection in brain DA neurons; [19]).

In brief, we suspect (being careful of taking into account the great complexity of the first motor disorder in human beings) that, at least in early stages of the disease there could be a possible proinflammatory situation enhanced by some kind of oxidative stress that would be deleterious for -among others- DA neurons in substantia nigra. Based on the evidences, we suggest that neurotrophic factors could protect the neurons under dysfunctional state. In this sense, $P_4$ could be in part responsible for stimulating such trophic factors; however, more studies become necessary to test this hypothesis. Actual efforts are underway to increase our understanding in this devastating disease.

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