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## REGULAR RESEARCH ARTICLE

# Morphine-Induced Dendritic Spine Remodeling in Rat Nucleus Accumbens Is Corticosterone Dependent

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## Abstract

**Background:** Chronic morphine treatments produce important morphological changes in multiple brain areas including the nucleus accumbens.

**Methods:** In this study, we have investigated the effect of chronic morphine treatment at a relatively low dose on the morphology of medium spiny neurons in the core and shell of the nucleus accumbens in rats 1 day after the last injection of a chronic morphine treatment (5 mg/kg once per day for 14 days). Medium spiny neurons were labeled with 1,1'-diiodo-3,3',3'-tetramethylindocarbocyanine perchlorate crystal and analyzed by confocal laser-scanning microscope.

**Results:** Our results show an increase of thin spines and a decrease of stubby spines specifically in the shell of morphine-treated rats compared with control. Since morphine-treated rats also presented an elevation of corticosterone level in plasma, we explored whether spine alterations induced by morphine treatment in the nucleus accumbens could be affected by the depletion of the hormone. Thus, bilaterally adrenalectomized rats were treated with morphine in the same conditions. No more alteration in stubby spines in the shell was detected in morphine-treated rats with a depletion of corticosterone, while a significant increase was observed in mushroom spines in the shell and stubby spines in the core. Regarding the thin spines, the increase observed with morphine compared with saline was lower in adrenalectomized rats than in nonadrenalectomized animals.

**Conclusion:** These results indicate that dendritic spine remodeling in nucleus accumbens following chronic morphine treatment at relatively low doses is dependent on corticosterone levels.

**Keywords:** morphine, nucleus accumbens shell, nucleus accumbens core, dendritic spines, adrenalectomy

## Introduction

Numerous data in the literature report that chronic treatments with psychotropic drugs produce changes in both brain and behavior that are distinct from their initial effects. Among the neuroadaptations usually observed, it is well known that drugs of abuse induce alterations in dendritic spine densities, observed in different brain structures, including the nucleus accumbens (Nac).

The Nac is mainly composed by GABAergic medium spiny neurons (MSNs) (Hjelmstad, 2004). MSNs are usually identified

by their dendritic arborization pattern but also by their high density of dendritic spines. These are key structures in the function of the central nervous system and essential components for neuronal connectivity and synaptic plasticity since they receive inputs from other regions. For example, MSNs received dopaminergic axons from the ventral tegmental area, connected to the spine neck, while the spine head is connected to glutamatergic inputs from prefrontal cortex

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## Significance Statement

Chronic morphine treatment is known to induce modifications in the morphology of medium spiny neurons, with alterations in dendritic spine densities. We found, in morphine treated rats once per day for 14 days at relatively low doses (5 mg/kg), that these modifications are specifically observed in the shell region of the nucleus accumbens and are spine type-specific, with an upregulation of thin spines whereas stubby spines were downregulated. Adrenalectomy reversed the alteration observed or revealed modification in spine densities, suggesting that the neuroplasticity observed is dependent on corticosterone levels.

(Smith and Bolam, 1990). Chronic morphine treatment induced an increase in dendritic spine density in frontal cortex and Nac (Pal and Das, 2013), whereas a 25% reduction in the size of dopamine neurons was observed in the ventral tegmental area (Sklair-Tavron et al., 1996). Morphine withdrawal also reduced dendritic branching and the number of dendritic spines in the Nac after long-term morphine withdrawal (Robinson and Kolb, 1999). Nac is a heterogeneous structure subdivided into functionally distinct regions termed core and shell that differ in their connectivity to other brain regions (Ito et al., 2004). Spiga and colleagues also demonstrated a selective reduction in dendritic spine density in Nac shell during spontaneous morphine withdrawal (Spiga et al., 2005).

A link between the hypothalamo-pituitary-adrenocortical axis and opioids is well known (Hayes and Stewart, 1985), and corticosterone levels have been shown to play a role in dendritic remodeling and spine density reorganization in MSNs of Nac (Morales-Medina et al., 2009). Thus, the goal of this study was to determine how chronic morphine treatments at relatively low doses would alter the 3 subtypes of dendritic spine (thin, stubby, and mushroom) in both the core and shell of the Nac, and to evaluate whether these neuroadaptations are corticosterone dependent by administering the same regimen of morphine treatment to adrenalectomized animals.

## Methods

### Animals

Male Sprague-Dawley rats (225–250 g, 6 weeks old, Janvier labs, Le Genest-Saint-Isle, France) were housed individually in standard laboratory conditions in a temperature- and light-controlled room (12-h-light/-dark cycle with lights on at 8:00 AM). Tap water and regular chow (Special Diets Services, Witham, Essex, UK) were provided ad libitum. Rats were acclimated to the animal facility and daily handled 1 week prior to the beginning of all experiments. Experiments were carried out in accordance with the European Communities Council Directive and were approved by the ethics committee.

### Chemicals

Paraformaldehyde (PFA) was purchased from Electron Microscopy Sciences (Hatfield, PA). Morphine was purchased from Francopia (Antony, France). Dulbecco's phosphate buffered saline (PBS) and 1,1'-diiododecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate were from Invitrogen (Cergy Pontoise, France), and Mowiol was obtained from Calbiochem (Nottingham, UK).

### Experimental Procedure

Following habituation (animals were daily handled 1 week), morphine was injected for 14 days via a s.c. route at 5 mg/kg, a dose known to induce a conditioned place preference behavior

in rats (Marie-Claire et al., 2007). A control group (NaCl 0.9%, s.c) was treated in the same conditions.

### Body Weight Measurements

During the treatment, weight gain was monitored every 24 hours throughout the saline and morphine treatment and the day after the last injection.

### Locomotor Activity

Locomotor activity was measured immediately after the last injection on day 14. The animals were taken from their housing cages, injected with saline or morphine, and immediately placed in the actimeter without previous habituation to this novel environment. Locomotor activity was evaluated just after the last injection of morphine during 26 hours in an actimeter (Imetronic, France) composed of 8 cages (34 × 21 × 19 cm) under low illumination (<5 lux) during the light periods and with a 12-h-light/-dark cycle with lights on at 8:00 AM. One rat was placed in each box to record its movements. Displacements were measured by photocell beams located across the long axis and above the floor. Vertical and horizontal activity was recorded and expressed in scores (mean ± SEM) as the total number of interruptions of the photocell beams. Their light/dark cycle was respected.

### Blood Collection and Determination of Corticosterone Levels

Rats were decapitated after lethal injection of pentobarbital, and trunk blood was collected into ethylenediaminetetraacetic acid-coated tubes (Greiner Bio One, Les Ulis, France) on day 15, 24 hours after the last injection. All blood samples were collected at the same time of day (9–10 AM), excluding a circadian rhythm effect. Blood was centrifuged at 2000 g for 10 minutes at 4°C, and plasma was collected and immediately frozen at –80°C until further analysis. Circulating corticosterone concentrations were assessed in duplicates using a commercially available kit (MP Biomedicals corticosterone rat/mouse). According to the manufacturer's protocol, the lowest analytical detectable level of corticosterone that can be distinguished from the Zero Calibrator is 4.1 ng/mL.

### Dendritic Spine Analysis

#### Surgeries

For surgeries, animals were anesthetized (ketamine 80 mg/kg /xylazine 10 mg/kg, i.p.) and then underwent bilateral adrenalectomy. After surgery, 0.9% of NaCl was added to the drinking water of adrenalectomized rats to maintain salt balance and to keep animals healthy. Rats were allowed to recover from the surgery for 1 week before the beginning of the chronic saline or morphine treatment. The success of the surgery was confirmed by the expected result of a body weight gain,

showing that adrenalectomized rats gain weight more slowly than nonadrenalectomized animals (Green et al., 1992; Bell et al., 2000; Scherer et al., 2011; Garcia-Perez et al., 2017).

#### Preparation of Brain Slices

Light fixation of brains with PFA was performed as previously described (Kim et al., 2007; Marie et al., 2012). Briefly, following anesthesia, tissues were fixed with intracardiac perfusion with ice-cold 1.5% PFA in 0.1 M phosphate buffer for 15 minutes with a peristaltic pump fixed at 20 mL/min. Brains were dissected and postfixed in 1.5% PFA in 0.1 M phosphate buffer for 1 h at 4°C and then transferred to phosphate buffered saline (PBS). Slices of 120  $\mu\text{m}$  containing the Nac were then collected in PBS using a vibratome (Leica).

#### Dendritic Spine Staining

The fluorescent lipophilic solid 1,1'-diocetadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate crystals were applied on the surface of the slice and were left at room temperature for 6 hours to allow dye diffusion along the neuronal membrane. Slices were then washed with PBS and fixed again in 4% PFA/PBS for 30 minutes. Then they were mounted on a glycerol-based mounting medium, Mowiol, to avoid shrinkage of dendritic structure caused by dehydration, containing DABCO as an antifade reagent.

#### Confocal Imaging of Dendritic Spines

Dendritic spines on MSNs in the Nac core and shell were imaged using Zeiss 510 confocal laser-scanning microscope. We used optimal settings pixel for frame size without zooming and the fluorescence was visualized with the 543-nm Helium/Neon laser. Serial stack images with step size ranging from 0.4 to 0.6  $\mu\text{m}$  were collected. The pinhole diameter was configured to 1 Airy unit (124  $\mu\text{m}$ ). Series stacks were collected from the bottom

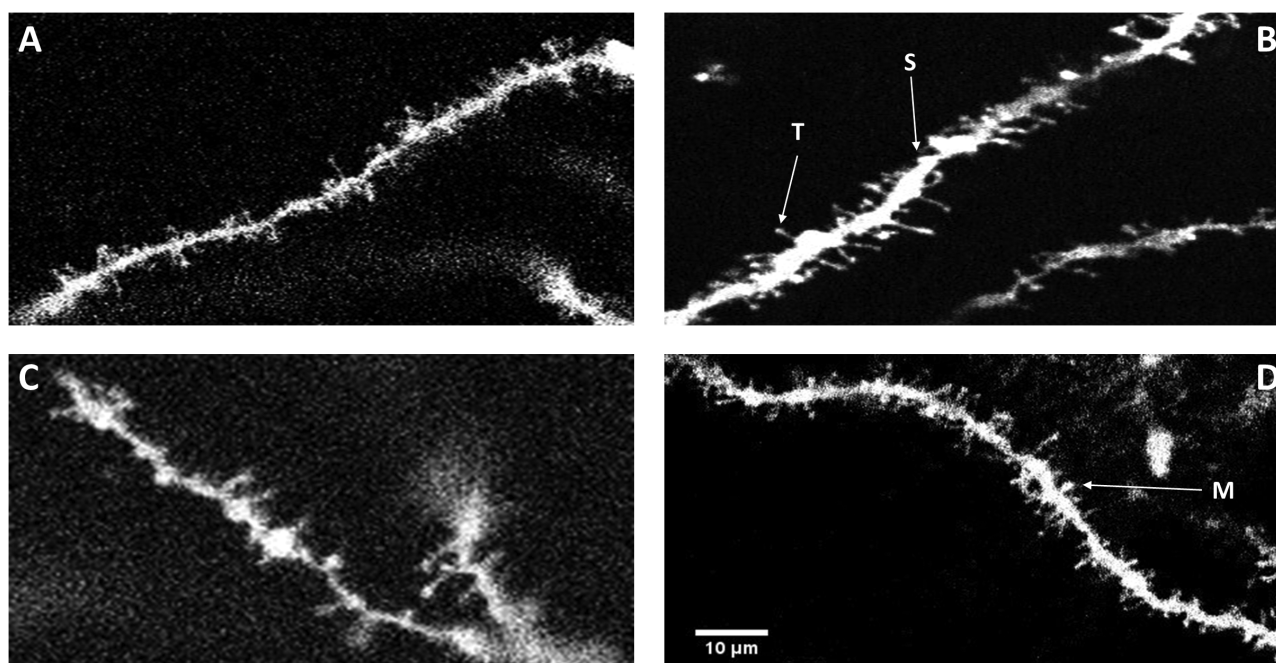
to the top, covering all dendrites with an optical slice thickness of 0.4 to 0.6  $\mu\text{m}$ . The resulting images (Figure 1) were then reconstructed to identify hidden protrusions according to Z-stack projections of the maximum intensity.

#### Image Analysis

Images were projected to reconstruct a 3D image using NIH ImageJ software (<http://rsbweb.nih.gov/ij/>). Dendritic protrusions (thin, stubby, and mushroom spines) were counted in the analysis according to their shapes: mushroom with large head and short neck; thin with thin head and long neck; and stubby with large head and no apparent neck. Filopodia were not included in the analysis, since these spines lack discernible heads and will not always give spines (Ziv and Smith, 1996). Only second-order dendrites on a length >50  $\mu\text{m}$  were analyzed. Two dendrites per neuron and 4 to 5 neurons for core or shell were analyzed in 5 to 8 animals per group. All measurements were performed by an experimenter blind to the conditions.

#### Statistical Analysis

Locomotor activity and body weight were analyzed by a 2-way ANOVA (time  $\times$  treatment) and the Bonferroni test was used as the posthoc test. Analyses of the amount of plasma corticosterone were performed with a Student's *t* test. Spine density was determined by summing the total number of each spine per dendritic segment length. These values were then averaged to yield the number of spines per micrometer for each animal. All data were expressed as mean  $\pm$  SEM. The results were analyzed using a Student's *t* test, comparing spine densities in the core and shell independently. Statistical tests were conducted with Graphpad prism 7 software. Values of  $P < .05$  were considered significant.



**Figure 1.** Digital reconstruction of dendritic segment of medium spiny neurons from nucleus accumbens (Nac) core (A, B) and shell (C, D) of morphine- (B, D) and saline- (A, B) treated rats. Images were obtained from morphine- or saline-treated rats on the first day of withdrawal. Brains were removed and fixed and dendritic spines were visualized using confocal microscope. Only contrast was slightly modified. Arrows indicate thin (T), stubby (S), and mushroom (M) spines.

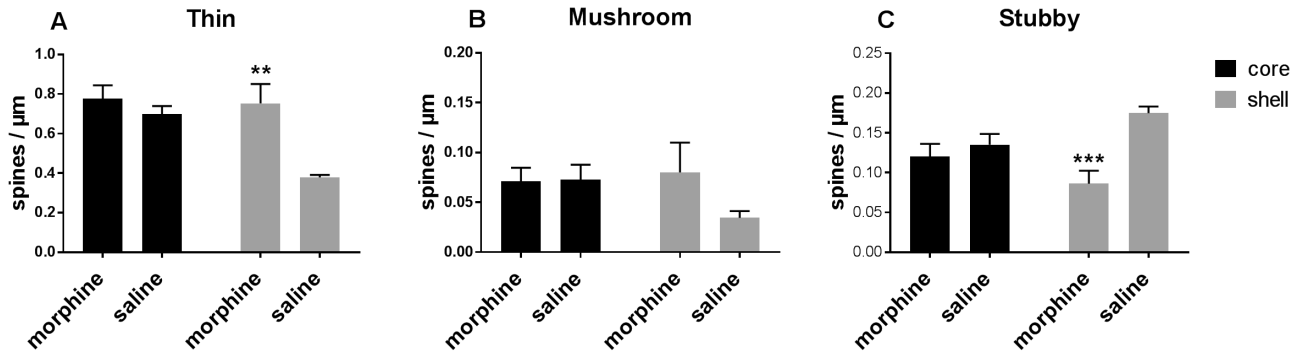


Figure 2. Rats received 14 days of morphine (5 mg/kg) or saline injections. On day 15, brains were collected and processed for dendritic spine analysis to measure thin (A), mushroom (B), and stubby (C) spine density. \*\* $P < .01$  \*\*\* $P < .001$  compared with saline, Student's *t* test ( $n = 5-7$  per group).

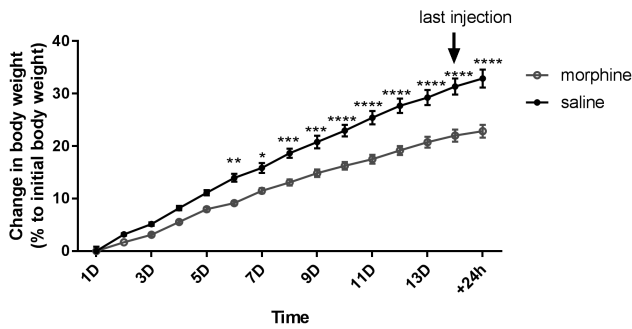


Figure 3. Evolution of body weight variation compared with weight before the first injection. The body weight of each rat was measured each day before the injection. Results are expressed as mean  $\pm$  SEM. Bonferroni test, \* $P < .05$ , \*\* $P < .01$ , \*\*\* $P < .001$ , \*\*\*\* $P < .0001$ ,  $n = 12$  rats per treatment.

## Results

### Effect of Morphine on Dendritic Spine Density in the Nac

We focused our attention on dendritic spine density 1 day after the last saline or morphine administration in Nac core and shell (Figure 1). Regarding thin spines (Figure 2A), Student's *t* test showed a significant increase of thin spines in morphine-treated rats compared with control, specifically in the shell [ $t(9) = 4.186$ ,  $P = .0024$ ], without effect in the core [ $t(11) = 1.061$ ,  $P = .310$ ]. For mushroom spines (Figure 2B), no significant effects were observed in both core and shell [ $t(11) = 0.075$ ,  $P = .942$  and  $t(9) = 1.626$ ,  $P = .138$ , respectively]. Finally, for stubby spines (Figure 2C), a specific decrease of these spines in the shell of morphine-treated rats compared with control was observed [ $t(9) = 5.122$ ,  $P = .0006$ ], without effect in the core [ $t(11) = 0.717$ ,  $P = .487$ ].

### Behavioral and Physiological Consequences of Morphine Treatments

#### Body Weight

All rats gained weight during our experiment but at a lower rate for morphine-treated rats. Rats receiving saline increased their body weight by 31% during the treatment whereas morphine-treated rats increased their weight by only 22%. Data for weight gain were analyzed by a 2-way (treatment  $\times$  time) ANOVA with time as a repeated measure (Figure 3). There was a significant interaction between treatment and time ( $F_{14,308} = 13.41$ ,  $P < .0001$ ), with an effect of time ( $F_{14,308} = 460.3$ ,  $P < .0001$ ) and treatment

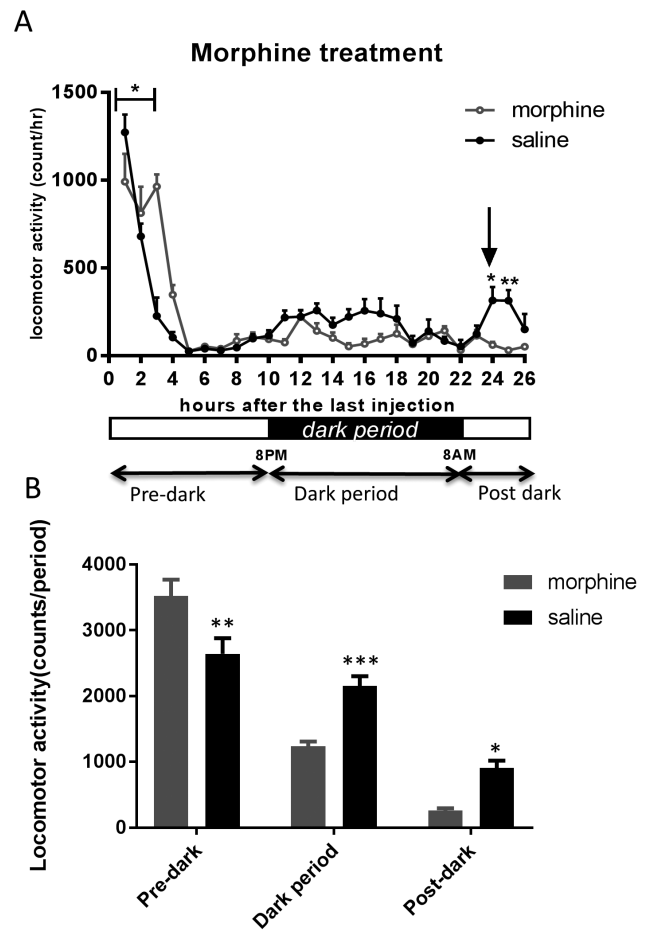


Figure 4. Rats received 14 days of morphine (5 mg/kg) or saline injections and locomotor activity was measured during 26 hours after the last injection. (A) Results are expressed in counts per hour (mean  $\pm$  SEM). \* $P < .05$ , \*\* $P < .01$ , Bonferroni test ( $n = 8$  per group). (B) Results are expressed in counts per period: pre-dark period (before the light is turned off), dark period (from 8 PM to 8 AM), and post-dark period (mean  $\pm$  SEM). \* $P < .05$ , \*\* $P < .01$ , Bonferroni test ( $n = 8$  per group).

( $F_{1,22} = 28.28$ ,  $P < .0001$ ). The Bonferroni posthoc test revealed significant differences in body weight gain between saline- and morphine-treated rats from the 6th day of the experiment.

In addition, body weight gain from the 1st day of chronic saline treatment to day 14 was reduced in adrenalectomized rats. Thus, the percentage of body weight gains were  $35.7\% \pm 0.7$  and



22.9%±2.4 in nonadrenalectomized and adrenalectomized rats, respectively ( $t(30) = 5.128, P < .0001$ ).

#### Spontaneous Locomotor Activity

The measure of locomotor activity showed differences between saline- and morphine-treated rats (Figure 4A). A 2-way ANOVA showed a significant treatment × time interaction ( $F_{(25,350)} = 6.638, P < .0001$ ), with a significant time effect ( $F_{(25,350)} = 41.55, P < .0001$ ) but no treatment effect ( $F_{(1,14)} = 2.405, P = .14$ ). In both groups, increased activity was observed immediately after placing animals in the actimeter. A significant effect of the morphine treatment on diurnal rhythms of locomotor activity was observed. Morphine-treated rats showed lower activity counts in the dark and early light period compared with controls, specifically at 10:00 AM ( $P < .05$ ) and 11:00 AM ( $P < .01$ ) (2 and 3 h after the light turns on, respectively) (Figure 4A).

Surprisingly, motor activity counts during the first hour after the final injection were actually greater in saline-treated rats than in the morphine group. This result may reflect novelty of the environment, as animals were not habituated to the actimeter chambers. It should be noted that this effect can only be observed over a very short period of time, and by cumulating the activities over the period until the light is turned off (daytime period), an increase in locomotor activity in morphine-treated animals is significant compared with saline animals (Figure 4B). Two-way ANOVA (period × treatment) revealed an interaction between the period and the treatment ( $F_{(2,28)} = 20, P < .0001$ ), with a main effect for the period ( $F_{(2,28)} = 133, P < .0001$ ) and no effect for the treatment ( $F_{(1,14)} = 2.405, P = .1432$ ). The posthoc test showed significant effects between saline and morphine groups in the pre-dark, dark, and post-dark periods (Figure 4B).

#### Corticosterone Level

Figure 5 illustrates plasma corticosterone levels measured on day 15, the day after the last injection of chronic morphine or saline treatment. Change in plasma levels was analyzed by a Student *t* test. A significant difference between morphine and saline-treated animals was detected (Figure 5;  $t(10) = 2.788, P = .0192$ ).

### Adrenalectomy Effects on Dendritic Spine Density

#### Impact of Adrenalectomy on Dendritic Spine Density in Control Rats

Firstly, we analyzed whether the adrenalectomies modified the dendritic spine densities in saline animals (Figure 6). Regarding

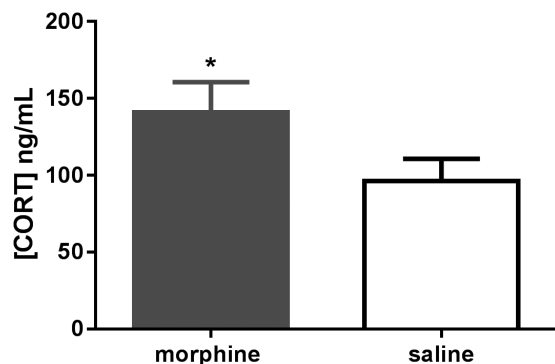


Figure 5. The plasma corticosterone (CORT) level was measured in morphine and saline control animals 1 day after the last injection of a chronic morphine or saline treatment. \* $P < .05$ , Student's *t* test ( $n = 6-8$  per group).

thin spines (Figure 6A), the Student's *t* test showed a significant decrease of thin spines in adrenalectomized rats, specifically in the core [ $t(13) = 2.902, P = .0124$ ], without difference in the shell [ $t(11) = 0.956, P = .360$ ]. For stubby spines (Figure 6C), a significant decrease in adrenalectomized rats was also observed, but specifically in the shell [ $t(11) = 2.287, P = .043$ ], without modification in the core [ $t(13) = 0.731, P = .477$ ]. Finally, no differences were observed between adrenalectomized and nonadrenalectomized rats in mushroom spine densities in both core and shell [ $t(13) = 0.251, P = .805$ , and  $t(11) = 1.407, P = .187$ , respectively] (Figure 6B).

#### Adrenalectomy on the Effects of Morphine on Dendritic Spine Density

These surprising data on opposite spine's subtype alteration in Nac shell following morphine treatment, in combination with a higher corticosterone level in plasma, necessitated an investigation of the neuroplastic changes following the same regimen of treatment but with a depletion of corticosterone by adrenalectomy. Thus, spine density was analyzed after either morphine or saline treatment in adrenalectomized animals in Nac core and Nac shell. Although morphine was able to reduce the stubby spine density in the NAc shell in nonadrenalectomized rats (Figure 2C), no differences were observed in adrenalectomized animals [ $t(11) = 0.152, P = .882$ ] (Figure 7C). In contrast, adrenalectomy was able to reveal morphine effects on the spine densities regarding mushroom spines in the shell [ $t(11) = 5.354, P = .0002$ ] (Figure 7B) and stubby spines in the core [ $t(13) = 2.441, P = .0297$ ] (Figure 7C). Regarding thin spines, although the increase of spine density was less important in morphine adrenalectomized rats compared to nonadrenalectomized animals, this effect was still significant in the shell [ $t(11) = 2.469, P = .0312$ ] (Figure 7A). No significant effect was observed in the Nac core [ $t(13) = 1.317, P = .2104$ ].

### Discussion

The main finding of this study is that with a history of chronic morphine treatment (14 days), dendritic spine remodeling in Nac shell observed 24 hours after the last administration is corticosterone dependent. In morphine-treated rats, thin spines are upregulated whereas stubby spines are downregulated in Nac shell without modification in the Nac core. Depletion of glucocorticoid reverses the alterations observed regarding stubby spines in the shell and reveals an effect on mushroom spines in the shell and stubby spines in the core, with an increase in spine density in morphine adrenalectomized rats compared with saline animals.

We found that dendritic spines were highly altered in the morphine group, specifically in the shell subregion, and surprisingly thin spines were upregulated by morphine whereas stubby spines were downregulated. There are conflicting results in the literature regarding the effect of morphine on density of dendritic spines. Reduction of second-order dendrites of accumbens shell during spontaneous (1 and 3 days) morphine withdrawal in rats has been reported with no effect in accumbens core (Kasture et al., 2009). Some other studies reported a global decrease of density of dendritic spines on MSNs after 1 month of morphine withdrawal (Robinson and Kolb, 1999; Robinson et al., 2002), but they did not distinguish between spine subtypes. Other authors found a global reduction in spine density in Nac shell in morphine-withdrawn rats, but the comparison was made between shell and core neurons in treated rats and not with a saline control group (Spiga et al., 2005; Diana et al., 2006). On

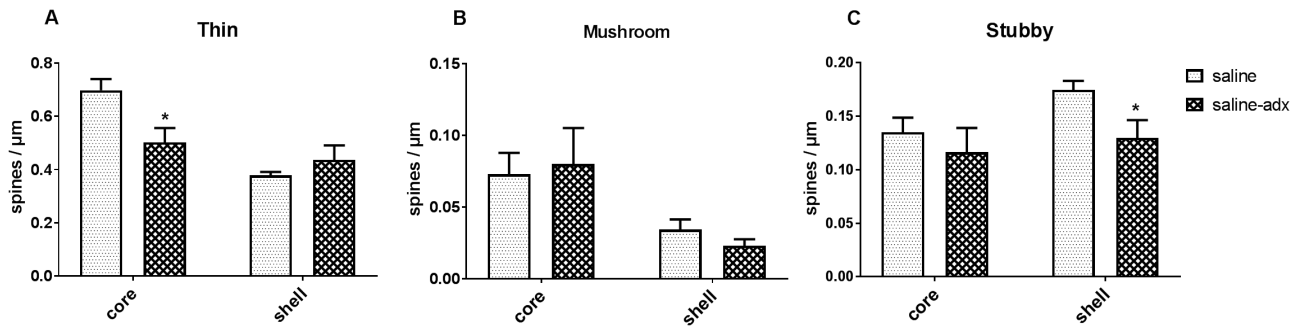


Figure 6. Adrenalectomized (saline-ADX) and nonadrenalectomized (saline) rats received 14 days of saline. On day 15, brains were collected and processed for dendritic spine analysis to measure thin (A), mushroom (B), and stubby (C) spine density. \* $P < .05$ , Student's  $t$  test ( $n = 6-8$  per group).

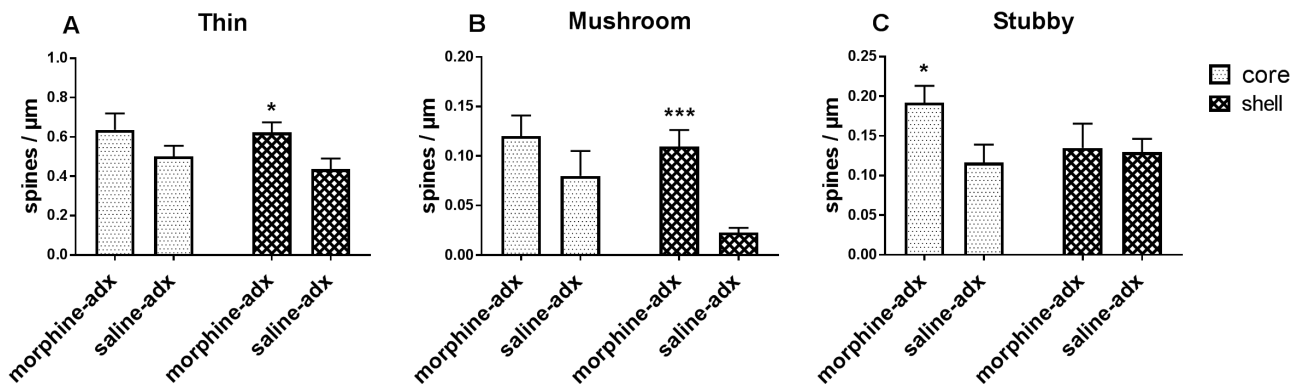


Figure 7. Adrenalectomized rats received 14 days of morphine (5 mg/kg) or saline injections. On day 15, brains were collected and processed for dendritic spine analysis to measure thin (A), mushroom (B), and stubby (C) spine density. \* $P < .05$ , \*\*\* $P < .001$ , Student's  $t$  test ( $n = 6-8$  per group).

the contrary, Graziane and collaborators reported no alteration of total spine density but with a significant increase of filipodia and a decrease of thin spines 1 day after morphine administration in Nac shell in mice (Graziane et al., 2016). Our results may seem contradictory to this latest study, but they used a 5-day repeated drug administration procedure, a protocol shorter than ours that can explain this discrepancy. Pal and Das (2013) reported a global 64% increase in dendritic spine density in Nac in morphine withdrawn mice compared with control. They further dissociated the type of spine but with a different methodology as they counted either mature spines or headless protrusions and thin spines as filipodia (Pal and Das, 2013). Thus, there is no clear consensus among the alterations of dendritic spine density during morphine withdrawal in Nac. Differences in experimental paradigm, doses, species, or the method of detection may explain the observed divergences.

The specific regulation observed in the shell part of the Nac may have several explanations. The shell part of the Nac is well known to play a key role in memory processes related to emotional events. Thus, as the animals were treated every day, during 14 days, exactly at the same time of the day, we could speculate that our animals were subjected to a Pavlovian conditioning (Geoffroy et al., 2014), which follows the general laws of learning and thus preferentially involved the shell part of the Nac (Di Chiara and Bassareo, 2007; Marie et al., 2012). Another hypothesis could be that the neuroadaptations observed could be the consequences of the repeated withdrawal periods in our animals, as we used a relatively low dose of morphine (5 mg/kg), with administration once a day, and a half-life of morphine in rats following subcutaneous administration of around 45 minutes (Miyamoto et al., 1988). During the 14 days of treatment,

animals certainly have a succession of positive and negative effects that could induce emotional states similar to a chronic stress (Chartoff and Carlezon, 2014). In good agreement with this hypothesis, rats treated with morphine showed a lower body weight gain compared with saline-treated rats. This loss of body weight was already reported by others with different regimens of morphine treatment (Martin et al., 1963; Yanaura et al., 1975; Desjardins et al., 2008).

We also studied the locomotor activity of animals for the 26 hours following the last injection. We observed alterations of locomotor activity in morphine-treated rats, with desynchrony in diurnal rhythms characterized by a lower activity counts in the late dark and very early light period compared with control animals. This alteration of locomotor activity may be interpreted as a "opioid dependence" (Van der Laan et al., 1991). This result could be expected as alterations in sleep-wake cycles have already been reported in both heroin self-administering rats and opiate addicts (Oyefeso et al., 1997; Coffey et al., 2016). Another adaptation usually observed following chronic morphine treatment and withdrawal is an increase in corticosterone levels (Kishioka et al., 1996), in good agreement with our results showing that morphine-treated animals have a higher basal plasma corticosterone level compared with saline animals.

It has been proposed that repeated morphine administration is a chronic stressor, as the hormonal and physiological effects of chronic morphine treatment are comparable with those observed with chronic stress (Houshyar et al., 2001; Chartoff and Carlezon, 2014). Furthermore, previous studies have demonstrated that increases in corticosterone levels induce morphological modifications in spine densities in several brain regions, including Nac (Morales-Medina et al., 2009). As we observed a

high plasma corticosterone level in morphine-treated rats compared with control, the next question was to consider to what extent removing corticosterone by adrenalectomy may impact dendritic morphology in morphine-treated rats. In this perspective, rats were bilaterally adrenalectomized and the success of this surgery was confirmed by the analysis of the body weight gain. Thus, between day 1 and day 14 of chronic treatments, adrenalectomized rats gained weight more slowly, as already reported (Green et al., 1992; Bell et al., 2000; Scherer et al., 2011; Garcia-Perez et al., 2017). In our experimental conditions, adrenalectomy reversed the alteration observed in stubby spines, but not in thin spines in the shell, and revealed effects of morphine on both the stubby spines in the core and mushroom spines in the shell. These results suggest a link between the hormone levels and dendritic spine densities in the Nac in morphine-treated rats. There is also a wide range of literature about the effect of corticosterone in dendritic spine density with different protocols. For example, corticosterone injections in rats for 21 consecutive days reduce branching of MSNs of Nac shell and result in decrease spine density compared with control animals (Morales-Medina et al., 2009). On the other hand, Kula et al. (2017) report that corticosterone administered twice daily for a shorter period of 7 days induces an increase in the number of thin dendritic spines on apical and basal dendrites in layer V pyramidal neurons of the primary motor cortex of rats, with no change in stubby or mushroom spines (Kula et al., 2017). An increase in dendritic arborization in basolateral amygdala is also described 12 days after an acute corticosterone treatment (Kim et al., 2014). Apart from exogenous corticosterone administration, chronic social defeat stress only increases stubby spines in Nac shell in susceptible mice, with no change in thin or mushroom spine density (Christoffel et al., 2011).

The opposite regulations observed in dendritic spine subtypes in the shell following chronic morphine treatments are quite surprising. Stubby spines and thin spines are both considered as immature subtypes whereas mushroom spines are more mature and stable (Duman and Duman, 2015). Wang et al. (2013) also found specific alteration of these spines and showed that a chronic corticosterone treatment induce changes in thin and stubby spines in hippocampus, without influencing mushroom spine density (Wang et al., 2013).

Dendritic spines can receive both glutamatergic and dopaminergic inputs. Indeed, cortical neurons make glutamatergic synapses on the head of dendritic spines of striatal MSNs while dopaminergic neurons exert control by synapsing on the neck of spines (Harvey and Lacey, 1997; Hashemiyoon et al., 2017). Some studies also report positive correlation between spine size and AMPA current (Matsuzaki et al., 2001). Thus, further studies should investigate the functional consequences of the regulation observed.

Altogether, the present findings highlight that chronic morphine treatment induces alterations in dendritic spine density, specifically in Nac shell, and that modifications in spine densities in the Nac are dependent on the corticosterone plasma level. Further studies are warranted to determine which specific molecular pathways are involved and what are the functional consequences of this plasticity.

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## Statement of Interest

None.

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