The glucocorticoid receptor in the nucleus accumbens plays a crucial role in social rank attainment in rodents

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Abstract

Social hierarchy in social species is usually established through competitive encounters with conspecifics. It determines the access to limited resources and, thus, leads to reduced fights among individuals within a group. Despite the known importance of social rank for health and well-being, the knowledge about the processes underlying rank attainment remains limited. Previous studies have highlighted the nucleus accumbens (NAc) as a key brain region in the attainment of social hierarchies in rodents. In addition, glucocorticoids and the glucocorticoid receptor (GR) have been implicated in the establishment of social hierarchies and social aversion. However, whether GR in the NAc is involved in social dominance is not yet known. To address this question, we first established that expression levels of GR in the NAc of high anxious, submissive-prone rats are lower than that of their low anxious, dominant-prone counterparts. Furthermore, virally-induced downregulation of GR expression in the NAc in rats led to an improvement of social dominance rank. We found a similar result in a cell-specific mouse model lacking GR in dopaminoceptive neurons (i.e., neurons containing dopamine receptors). Indeed, when cohabitating in dyads of mixed genotypes, mice deficient for GR in dopaminoceptive neurons had a higher probability to become dominant than wild-type mice. Overall, our results highlight GR in the NAc and in dopaminoceptive neurons as an important regulator of social rank attainment.

Keywords

Social dominance

Anxiety

Dopaminoceptive neurons

Rats

Transgenic mice

1. Introduction

Social rank influences behavior and physiology in both humans and animals, and has been linked with the development of psychopathologies (Allan and Gilbert, 1997; Sapolsky, 2005). Most social species are organized in social hierarchies. The organizing principle of social hierarchies is to provide dominant individuals with priority access to resources, such as new territory, food, water and mating partners (Broom, 2002; van der Kooij and Sandi, 2015).

In laboratory rodents, social hierarchy is often established through repeated agonistic and antagonistic or aggressive interactions. It usually develops within a few days and remains stable over long periods of time (Blanchard et al., 1988). The reward- and motivation-related mesolimbic dopamine system has been identified to be important for the establishment of social dominance [reviewed in (Ghosal et al., 2019)]. According to previous observations in rodent models for trait anxiety, the nucleus accumbens (NAc) is actively engaged during aggressive encounters (Beiderbeck et al., 2012). In humans, imaging studies have demonstrated activation of the NAc when confronted to social status or social competition (Ly et al., 2011; Zink et al., 2008). Chester and colleagues (2016) also reported a greater activation of the NAc following provocation in a behavioral aggression task (Chester and DeWall, 2016). Furthermore, our lab previously showed that inactivation of the NAc reduces social dominance in rats (Hollis et al., 2015). In addition, we observed that dopaminoceptive D1 receptor-containing neurons (but not other cell types) in the NAc are activated by social competition, particularly in dominant-prone individuals (Hollis et al., 2015; van der Kooij et al., 2018), while blockade of D1 receptors diminishes social dominance (van der Kooij et al., 2018). Altogether, previous work supports an important role for the NAc, and more specifically for dopaminoceptive neurons in the NAc, in the establishment of social rank.

Several lines of evidence indicate that stress response and more particularly glucocorticoid hormones may also play a key role in the establishment of hierarchy. We have previously shown that acute stress increases the propensity to become subordinate in a pair of unfamiliar rats matched for age, body weight and anxiety levels (Cordero and Sandi, 2007). Glucocorticoids levels rise in the bloodstream

upon social defeat (Barik et al., 2013), following social encounters (Bronson and Eleftheriou, 1965; Schuurman, 1980) and have been recently shown to participate to the establishment and maintenance of social hierarchies (Timmer and Sandi, 2010; Weger et al., 2018). Importantly, selective inactivation of the gene encoding the glucocorticoid receptor (GR) in dopamine-innervated brain regions (including the striatum, the NAc and the deep cortical layers) was shown to prevent social aversion in animals undergoing social defeat (Barik et al., 2013). Altogether these data indicate that glucocorticoids through GR might play a key role in shaping behavioral trajectories leading to social rank attainment. However, it is not yet known whether GR in the NAc is important for the modulation of social hierarchies.

In this study, we investigated the potential role of GR in the NAc and dopaminoceptive neurons in rank attainment. To this end, we first analyzed expression levels of GR and mineralocorticoid receptor (MR) in the NAc in high and low anxious rats that are, respectively, prone to become submissive or dominant, when submitted to dyadic encounters in competition for a new territory. Next, we explored the causal involvement of GR in rank attainment through adeno-associated virus (AAV)-induced GR downregulation in the NAc of rats. Finally, in order to address GR involvement in a cell type specific manner, we investigated rank attainment in dyads of cohabitating mice involving one wild-type mouse and a mouse lacking GR in dopaminoceptive neurons.

2. Materials and Methods

2.1 Animals

Adult male Wistar rats (Charles River) weighing 250-275g at the start of experiments were used. For the initial experiments that examined social hierarchy between high and low anxious animals, rats were singly housed upon arrival to the vivarium to avoid any influence from social experiences. For all other experiments, after arrival, animals were housed 2 per cage and allowed to acclimate to the

vivarium for one week. All animals were subsequently handled for 2 minutes for a minimum of 3 days. They were weighed upon arrival as well as weekly to ensure good health.

Mouse experiments were conducted in adult weight-matched (approximately 25g at the start of the experiments) male GR^{D1Cre} mice and their control littermates bred in a C57Bl/6 background. The generation of GR^{D1Cre} mice has been described previously (Ambroggi et al., 2009). Briefly, Nr3c1 (*GR*) gene inactivation was selectively targeted in dopaminoceptive neurons ($Nr3c1^{loxP/loxP}$;(Tg)D1aCre (Lemberger et al., 2007) hereafter designed GR^{D1Cre}). Experimental animals were obtained by mating $Nr3c1^{loxP/loxP}$ females with $Nr3c1^{loxP/loxP}$;Tg:D1aCre males.

Rats and mice were maintained under standard housing conditions on a 12h light-dark cycle (lights on at 7:00 AM). Food and water were available ad libitum. Experiments on rats were performed with the approval of the Cantonal Veterinary Authorities (Vaud, Switzerland) and carried out in accordance with the European Communities Council Directives of 24 November 1986 (86/609EEC). Experiments on mice were performed in accordance with French Ministère de l'Agriculture et de la Forêt (87-848) and the European Directive 2010/63/UE and the recommendation 2007/526/EC for care of laboratory animals.

2.2 Experimental design

One week after their arrival, rats were tested in an elevated plus maze to assess basal anxiety and were classified as high (HA) or low anxious (LA), according to the amount of time spent in the open arms (HA, ≤5% open arm duration, LA ≥20% open arm duration) (for details, see Supplementary Materials and Methods). HA and LA animals were then matched in pairs according to their body weight and tested for social hierarchy. A separate cohort of HA and LA animals was sacrificed without hierarchy testing to extract brains for gene expression analysis.

For the viral downregulation of GR, animals were assigned to experimental groups with averaged anxiety levels being similar between the groups. Rats then underwent surgery for delivery of a viral construct expression shRNA targeting GR (GR-KD) or a viral construct expressing a scrambled control

(SCR) in the NAc. After 6 weeks of recovery, animals' anxiety levels and locomotion were tested in an open field. SCR and GR-KD rats were then matched by weight and paired in a new cage, avoiding previous cage mates to be placed together. After two weeks of cohabitation, the social confrontation tube test was performed. Seventy-two hours later, they were sacrificed and their brains were extracted and used to assess virus localization and efficiency.

For the experiment involving GR mutant mice, 12 weeks old control and GR^{D1Cre} mice were matched by body weight and paired in a new cage. As for the rat experiments, each mouse from a given pair was from a different litter and hence were not previously cage mates. After one week of cohabitation, social dominance was assessed in the dyad using the social confrontation tube test. Seventy-two hours later, mice were sacrificed for basal corticosterone analysis.

2.3 Open field and novel object reactivity tests

The open field (OF) and novel object reactivity (NOR) tests were performed to assess the rats' emotional and exploratory/locomotive behavior, as well as their reactivity upon novelty exposure. The tests were performed as previously described (Kohl et al., 2013). Briefly, a circular open arena was utilized (1 m diameter, 40 cm high). Each rat was placed near the wall and it was allowed to explore the apparatus freely for 10 minutes. The floor of the open field was virtually divided in three parts: a center zone in the middle of the arena with a diameter of 25 cm, an intermediate zone with a diameter of 75 cm and the remaining wall zone along the walls of the arena. At the end of the 10-minute period, an object (yellow plastic bottle) was introduced into the center of the arena for the NOR test. Each animal was allowed to explore the arena and object for 5 minutes. The time spent in each zone, the total distance moved and the time spent sniffing the object were recorded. The apparatus was cleaned with 5% ethanol solution and dried thoroughly between each animal.

2.4 Social dominance tests

Dominance was assessed using the social hierarchy test or the social confrontation tube test (Wang et al., 2011; Zhou et al., 2018, 2017). The social hierarchy test was performed as described previously (Hollis et al., 2015). Rats were pair-wise matched for body weight but animals in each pair were of opposite anxiety profile. Animals were marked on their body for identification and placed in pairs in a new (neutral) cage without food or water for 20 minutes. During the social hierarchy test both rats displayed spontaneously offensive behavior, but this balance typically shifted in a favor of one animal towards the end of the test. Offensive behavior typically exhibited by a dominant animal in a territorial situation (Koolhaas et al., 1980) were quantified in terms of duration, including the : offensive upright, lateral threat and keeping down postures (see also Fig. 1A). The cumulative duration of these behaviors was summed to provide a measure of total offensive behavior. Pairs in which offensive behavior was virtually absent during social encounter (no rat displaying > 10s of total offensive behavior) were excluded from the analysis, as the relative social dominance in these pairs cannot be reliably measured. In total, 5 pairs were excluded due to low fighting behavior.

The protocol of the social confrontation tube test was adapted from Wang et al. (2011). For the rat experiments, the tube test was performed in dyads that had been living together for 2 weeks. Each rat was individually trained to move forward out of a clear Plexiglas tube (diameter, 7.5 cm; length 100 cm) over five consecutive days, with two trials per day. Each rat of the dyad learned to move forward only from one end. The size of the diameter is just sufficient to permit an adult rat to move through the tube without reversing its direction. If the rat retreated or stopped moving for a certain amount of time, it was gently pushed by touching its tail with a plastic stick. The tube was cleaned and dried between each trial with 5% ethanol to remove odor, urine or feces. After five days of training, dominance was evaluated for five consecutive days. The two rats of each dyad were handled and guided simultaneously at the opposite ends of the tube until they entered the tube and reached the middle part. The time spent in the tube was recorded until one of the two rats forced its cage mate to

go backwards and exit the tube. The rat that retreated from the tube was designated as the 'loser' of that trial and was considered to be the subordinate rat, whereas the rat that won four trials or more designated as the 'winner' of the trial and was considered as the dominant rat.

The tube test protocol on mice was similar to that performed in rats with minor modifications: the dyads had been living together for one week before testing, the apparatus was of a smaller diameter and length (diameter, 3cm; length 30cm) and mice were trained over seven consecutive days, with three trials per day. The dominance was then evaluated for three consecutive days, with three trials per day. The mouse that won seven trials or more was the dominant and the loser was the subordinate mouse.

2.5 Viral downregulation of GR

Two weeks after arrival, rats were subjected to surgery for the viral downregulation of GR in the NAc wherein an adeno-associated AAV1/2 vector containing an U6-pm-GR3 shRNA-terminator-CAG-EGFP-WPRE-BGH-polyA-expression cassette was used. Control animals were injected with a scrambled shRNA construct (AAV1/2-U6-SCR shRNA-CAG-EGFP-WPRE-BGH-polyA). All viral constructs used were designed and produced by GeneDetect, New Zealand. The vector incorporated the following regulatory elements: rAVE^{TM} construct containing EGFP, the hybrid chicken B-actin/CMV enhancer (CAG) promoter region, a cis-acting woodchuck post-transcriptional regulatory element (WPRE) and a bovine growth hormone polyadenylation sequence (BCG-polyA). A perfect match (pm) short hairpin RNA (shRNA) construct against GR, driven by U6 promoter was also incorporated. For the scrambled vector, the same backbone without the cDNA was used. Initially, the animals were anaesthetized by inhalation of isoflurane (5% oxygen-isoflurane mixture) and installed in a stereotaxic frame to avoid any head movements during the surgical procedure. To target both the core and the shell of the NAc , two injections sites were used with the following coordinates (Paxinos and Watson, 2006): 1.3 and 2.5 mm posterior to bregma, 1.0 and 1.5 mm from midline, 7.0 ventral from skull. A volume of 0.8 µl of either GR-KD or scrambled vector ($1.3x10^{12}$ viral genomic particles/ml) was bilaterally injected in the

NAc with a constant flow rate of 0.1 µl/min. The injectors were left in site for 5 minutes after the end of the actual injection. After removing the injectors, animals were treated with paracetamol (500 mg/700 ml H2O, Dafalgan, Bristol-Myerts Squibb, Agen, France) via the drinking water for seven days after the surgery. The animals were allowed to recover for 6 weeks from surgery so that the maximum viral expression could be reached before the behavioral testing was started. Successful viral knockdown was verified by gene expression analysis, immunofluorescence and immunohistochemistry (see Supplementary Materials and Methods for more details).

2.6 Corticosterone analysis

Trunk blood was collected during sacrifice for basal corticosterone measurement in both rats and mice. Animals were sacrificed in the morning. Free corticosterone measurements were obtained from blood plasma samples via centrifugation and subsequent measurement using an enzymatic immunoassay kit that was performed according to manufacturer's instructions (Enzo Life Sciences, Switzerland). Levels were calculated using a standard curve method.

2.7 Statistical analysis

Data were analyzed with Student *t*-test or Mann-Whitney test, as appropriate, using the statistical package GraphPad Prism 5 (GraphPad software Inc., USA). If Levene's test for equality of variances was significant, equal variance was not assumed and the altered degree of freedom was rounded to the nearest whole number. Dominance in the tube test was analyzed with Fisher's exact test. All bars and error bars represent the mean ± SEM. Significance was set at $p < 0.05$, while the *p*-values were considered tending toward significance when $0.05 \leq p \leq 0.1$. Graphs were created using GraphPad Prism 5.

3. Results

3.1 Submissive-prone rats show reduced GR gene expression in the NAc

As reported previously (Hollis et al., 2015; van der Kooij et al., 2018), high anxious rats had a higher probability to lose a competition for a new territory when confronted with low anxious counterparts. To understand if the outcome of the social competition could be considered as a phenotypic trait, we analyzed the hierarchical behavior across numerous cohorts of high and low anxious animals. Qualitative analyses of offensive behaviors indicated that high anxious rats exhibited reduced offensive behavior both for the compound index (Figure 1b; $U = 1861$, $p < 0.0001$) and individually, for each of the respective offensive behaviors (Figure 1a, b; offensive upright: $U = 2741$, $p < 0.0001$; keeping down: $U = 2308$, $p < 0.0001$; lateral threat: $U = 2912$, $p < 0.0001$). Therefore, we consider high anxious animals as submissive-prone (subP) and low anxious rats as dominant-prone (domP) animals.

We next assessed the expression levels of GR and MR genes in the NAc in an independent group of rats, which were only tested for anxiety levels. We found significantly lower GR gene expression in the NAc of subP rats compared to domP conspecifics (Figure 1c; $t_7 = 2.93$, $p < 0.05$). In contrast, MR expression was similar for both groups (Figure 1d; $t_7 = 0.45$, $p = 0.67$). Interestingly, basal corticosterone levels were higher in subP rats than in domP animals (Figure S1).

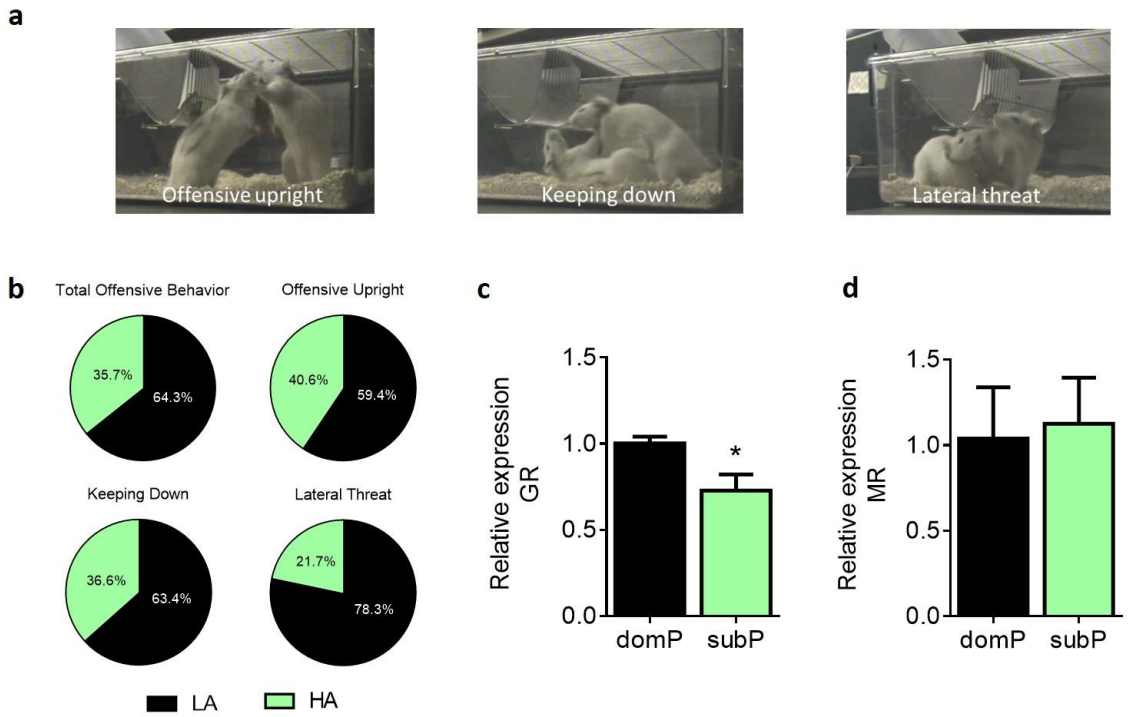

Figure 1. Characterization of dominant and submissive-prone rats in behavior and gene expression. Example of rats illustrating the three dominant postures considered in the social hierarchy test: offensive upright, keeping down and lateral threat **(a)**. High anxious (HA) rats exhibited lower percentage of total offensive behavior, offensive upright, keeping down and lateral threat in a social hierarchy test than low anxious (LA) animals **(b)**. GR expression analysis in the nucleus accumbens (NAc) revealed lower expression levels in submissive-prone (subP) rats in comparison to dominant-prone (domP) animals **(c)**. The mRNA levels of MR were similar between groups. N: LA/domP rats = 97 (b) or 4-5 (c, d) and HA/subP rats = 97 (b) or 4-5 (c-d). *p < 0.05, vs domP. Results are expressed as mean ± SEM.

3.2 Downregulation of the GR in the NAc modifies spontaneous behaviors in novel environments

In order to investigate whether GR in the NAc plays a role in social competition, we injected an adeno-associated virus downregulating GR (GR-KD) or, in the control group, a virus expressing a scrambled construct (SCR) into the NAc. Immunofluorescence analyses confirmed that the virus was mainly expressed in the medial part of the NAc core and shell (Figure 2a). Knockdown efficiency in the NAc was then confirmed through qRT-PCR quantification of GR mRNA levels (Figure 2b; $U = 4.00$, $p < 0.05$) and by immunostaining (Figure 2c).

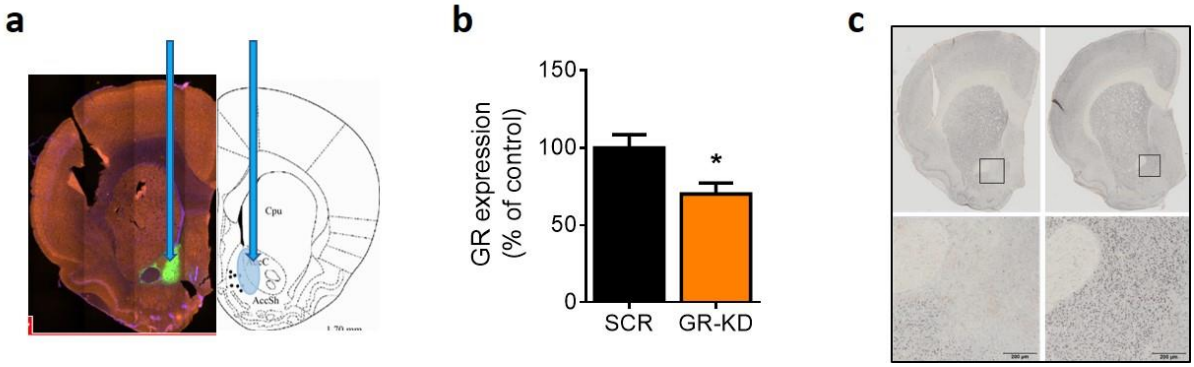

Figure 2. Verification of viral GR downregulation in the NAc. Targeted area in the NAc and GFP localization with 4x magnification of the NAc are shown in **(a)**. GR mRNA expression in the NAc was decreased in rats injected with the AAV-U6-shGR virus (GR-KD) compared to scrambled (SCR)-infused rats **(b)**. DAB immunohistochemistry (40x magnification) further confirmed the downregulation of GR in the NAc in GR-KD animals (left panel) compared with SCR rats (right panel) **(c)**. *N:* SCR = 6; GR-KD = 6. *$p < 0.05$, vs SCR. Results are expressed as mean ± SEM.

Six weeks after the virus injection, anxiety-like and exploratory behaviors were assessed in the OF and NOR tests. In the OF test, GR-KD rats spent a higher percentage of time in the center (Figure 3b; $U = 44.00$, $p < 0.05$) and in the intermediate zone (Figure 3c; $U = 31.00$, $p < 0.01$) and less time in the wall area (Figure 3d; $t_{25} = 3.10$, $p < 0.01$) than SCR rats. The distance traveled during the test was significantly longer in GR-KD animals (Figure 3e; $t_{25} = 3.66$, $p < 0.01$). Similarly, in the NOR test, GR-KD rats spent more time in the center (Figure 3f; $U = 44.00$, $p < 0.05$) but less time in the wall area (Figure 3h; $t_{25} = 2.44$, $p < 0.05$) than SCR animals. No significant difference was found in the time spent in the intermediate zone (Figure 3g; $U = 58.00$, $p = 0.12$). Moreover, there was a trend for GR-KD rats to move greater distance than SCR-infused controls (Figure 3i; $t_{25} = 1.83$, $p = 0.08$). Finally, GR-KD rats spent more time sniffing the novel object than SCR conspecifics (Figure 3j; $U = 47.00$, $p < 0.05$).

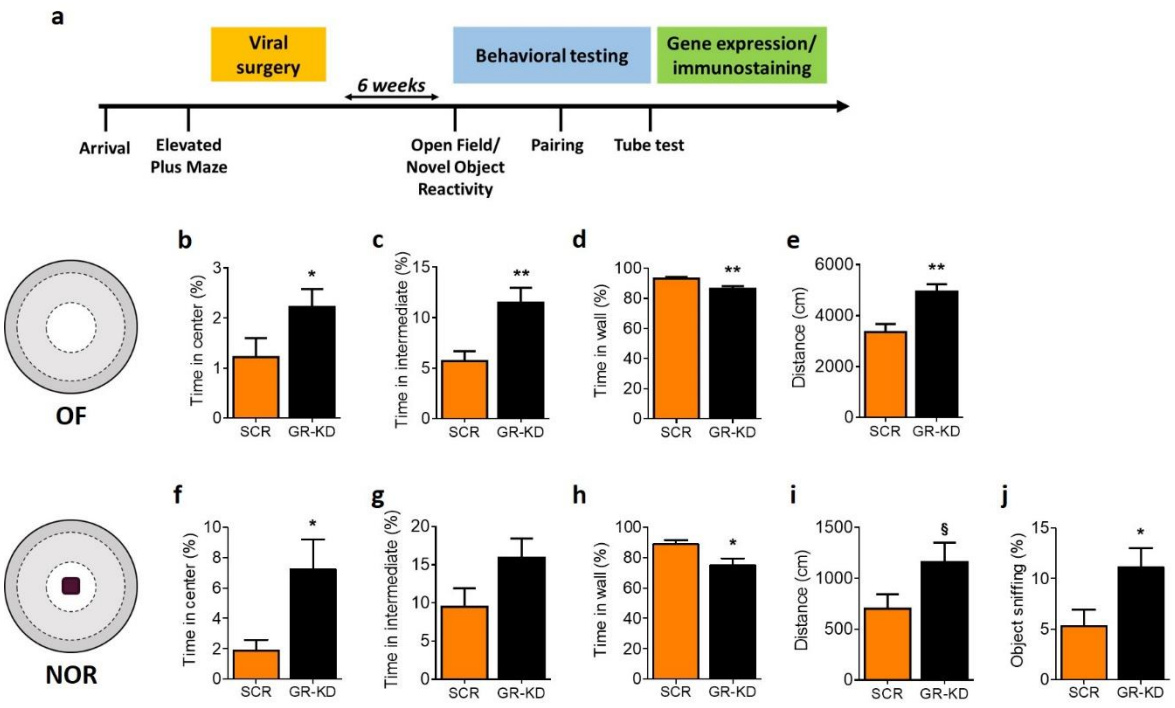

Figure 3. GR knockdown in the NAc reduces anxiety-like behavior in the open field and novel object reactivity test. Experimental design **(a).** GR-KD rats spent more time in the center **(b)** and the intermediate zone **(c)** of the open field (OF) and reduced time in the wall zone **(d)** compared to SCR animals. The distance traveled was higher in GR-KD rats **(e)**. Similarly, GR-KD rats spent more time in the center when a novel object was presented (NOR) **(f)** and less time in the wall zone **(h)** than SCR animals. No significant difference was found in the time spent in the intermediate zone **(g).** The distance moved tended to be higher in GR-KD rats when the object was present **(i)**. Furthermore, GR-KD animals spent more time sniffing the object than SCR rats **(j)**. *N:* SCR = 12; GR-KD = 14-15. *$p < 0.05$, **$p < 0.01$, §$p < 0.1$, vs SCR. Results are expressed as mean ± SEM.

3.3 Downregulation of the GR in the NAc promotes social dominance

Animals were matched for body weight and placed together to cohabitate in a new cage in dyads, involving one animal from each group (i.e., GR-KD and SCR rats). Two weeks after the beginning of their cohabitation they underwent the social confrontation tube test (Figure 4a). Social rank was considered stable when rats' performance yielded the same rank for four consecutive days (see Figure 4b for an example from a single cage, Figure 4c for the average of the 12 cages). GR-KD rats had a higher number of winning trials, indicating that they exhibited more dominance than SCR animals

(Figure 4d; Fisher's exact test, $p < 0.05$). In the winning trials, the latency to push the opponent out of the tube was similar for both groups (Figure 4e; $U = 12.00$, $p = 0.52$). No group difference in basal corticosterone levels was detected (Figure 4f; $U = 142.00$, $p = 0.73$).

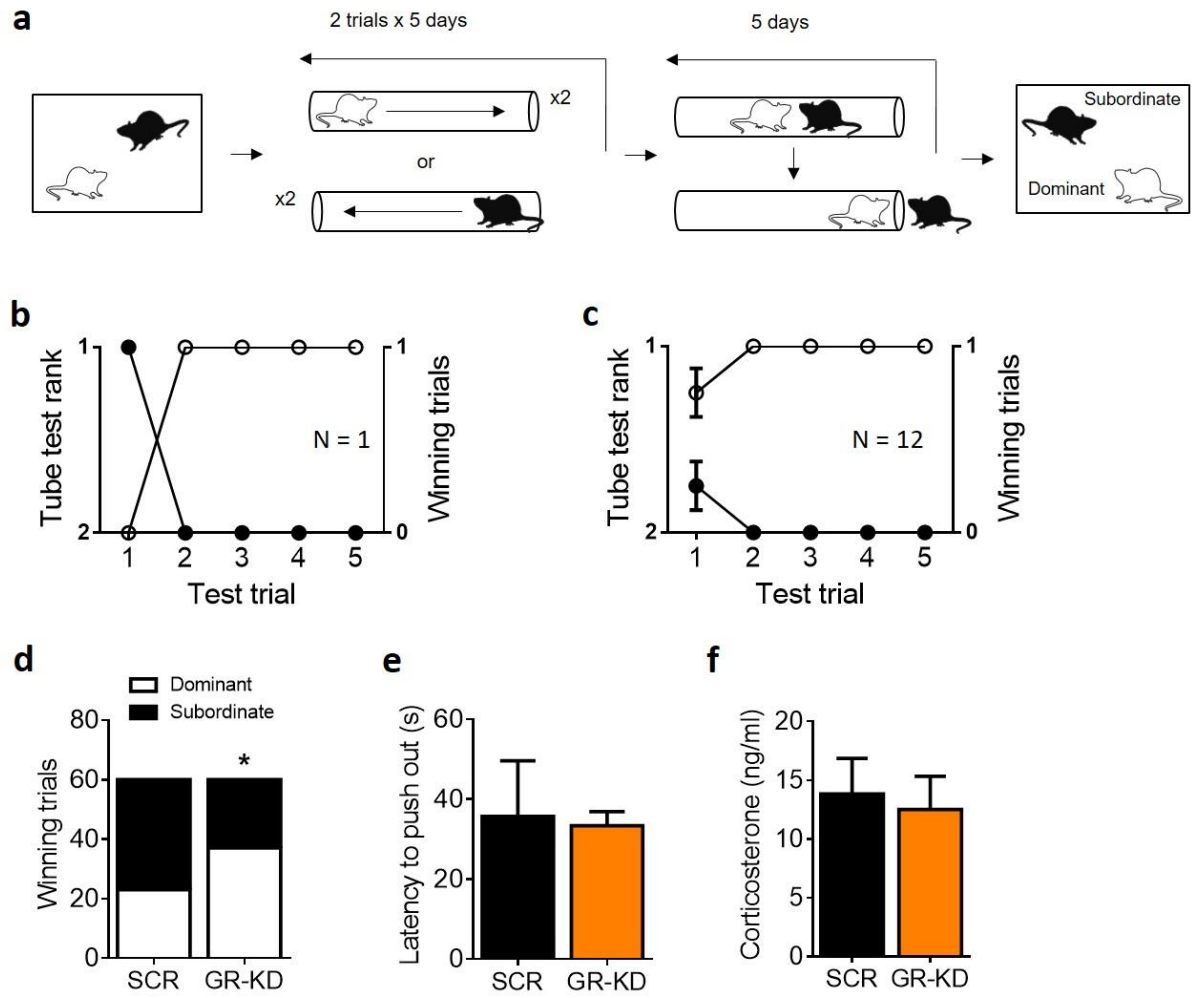

Figure 4. **GR knockdown in the NAc enhances social rank attainment in a dyadic hierarchy in rats.** Following dyadic cohabitation of a GR-KD and a SCR-infused rats for two weeks, each dyad of rats was tested for dominance using the social confrontation tube test **(a)**. Example of one cage representing the rank and winning trials as function of tube test trials **(b)**. Summary for twelve cages over the 5-day test trials **(c)**. GR-KD rats had a higher number of winning trials than SCR animals **(d)**. The latency to push the opponent out of the tube was similar between groups **(e)**. Basal corticosterone measurement at sacrifice was not significantly different between groups **(f)** *N:* SCR = 12, GR-KD = 12. *$p < 0.05$, vs SCR. Results are expressed as mean ± SEM.

3.4 GR gene deletion in dopaminoceptive neurons promotes social dominance in mice

Next, we assessed whether the GR knockout specifically in dopaminoceptive neurons affects the emerging social status in mouse dyads. To this end, dyads constituted by one control and one GR^{D1Cre} mice were formed. One week afterwards, the emerged social hierarchy was tested with the social confrontation tube test (Figure 5b). We performed two replication experiments (see figure S4 for the detailed results of each experiment), and data presented here are pooled. We considered social rank as stable when mice performance yielded the same rank for more than seven consecutive trials (see Figure 5c for an example from a single cage, Figure 5d for the average of all tested pairs). Note that the threshold is different from the one in rats, according to a protocol developed in our lab on the model described by Wang and colleagues (Wang et al., 2011). GR^{D1Cre} mice won the tube contest more times and, therefore, were considered to be more dominant than controls (Figure 5e; Fisher's exact test, $p < 0.001$). In the winning trials, there were no group differences in the latency to push the opponent out of the tube (Figure 5f; $U = 953.50$, $p = 0.67$). Basal corticosterone levels were similar between groups (Figure 5g; $U = 41.50$, $p = 0.22$).

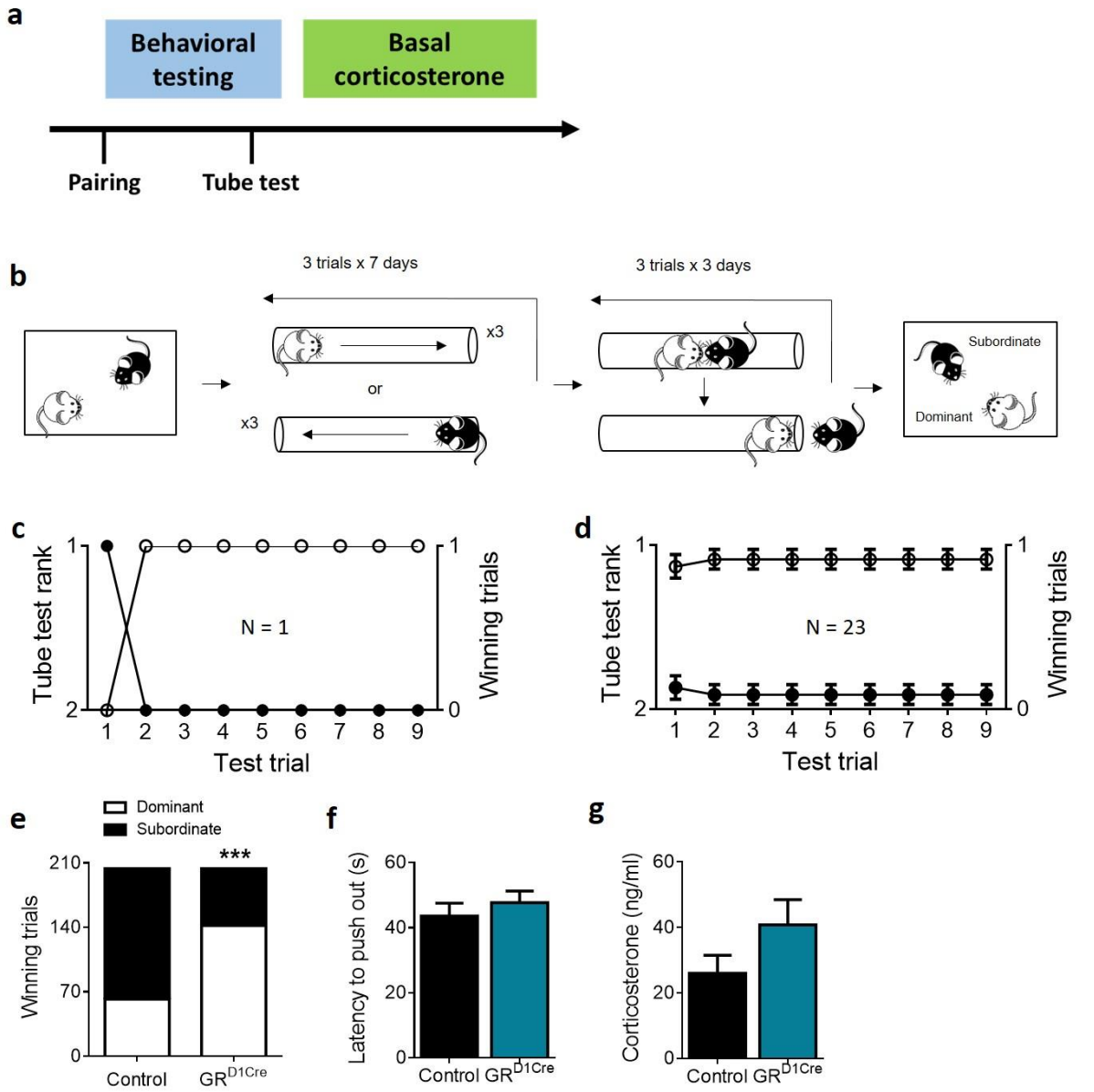

Figure 5. **Impact of GR gene inactivation in dopaminoceptive neurons on social rank attainment in a dyadic hierarchy.** Experimental design **(a)**. Following cohabitation of a dyad consisting of a control and a GR^{D1Cre} mouse for one week, each dyad of mice was tested for dominance using the social confrontation tube test **(b)**. Example of one cage representing the rank and winning trials as function of tube test trials **(c)**. Summary for 23 cages over the 3-day test trials **(d)**. GR^{D1Cre} mice had a higher number of winning trials than control animals **(e)**. The latency to push the opponent out of the tube however did not differ **(f)**. Finally, basal corticosterone levels measured at sacrifice were similar between groups **(g)**. N: Control = 23 (e) or 11 (f, g), GRD1Cre = 23 (e) or 11 (f, g). ***$p < 0.001$, vs control. Results are expressed as mean ± SEM.

4. Discussion

Stress and glucocorticoids have been shown to be important modulators for the long-term establishment of social hierarchies -specifically, for social subordination- in rodents (Cordero and Sandi, 2007; Timmer and Sandi, 2010; Weger et al., 2018). However, these studies have not addressed whether stress or glucocorticoids modulate social dominance via the GR or the MR, nor where in the brain these effects occur. In this study, we detected decreased gene expression of GR, but not MR, in the NAc of high anxious animals that are more prone to lose a social competition and become subordinate. Thus, we investigated the role of GR for social dominance. We observed that knocking down GR in the NAc led to decreased anxiety and made rats more prone to win the contests in the tube test. Furthermore, a similar result was found in another model in which GR is inactivated within dopaminoceptive neurons including the NAc. Indeed, GR^{D1Cre} mice exhibited more dominant behavior than wild-type mice in the tube test. Overall, our results highlight GR in the NAc and in dopaminoceptive neurons as an important regulator for social rank attainment. Our observations are in line with previous studies reporting a prominent role of the NAc in the development and/or expression of social dominance in rodents (Anstrom et al., 2009; Beiderbeck et al., 2012; Fantin and Bottecchia, 1984; Hollis et al., 2015; Puciklowski et al., 1988). Recently, our laboratory has shown that the social competition for a new territory between outbred Wistar rats leads to NAc activation, and that transient pharmacological inhibition of the NAc with the $GABA_A$ agonist muscimol results in reduced social competence (Hollis et al., 2015). Similarly, neuroimaging studies in humans found NAc activation under tasks involving manipulations of social status (Bouc and Pessiglione, 2013; Ly et al., 2011) or social competition (Zink et al., 2008). Moreover, the NAc has been described as a key player in the expression of motivated behavior (Salamone et al., 2015; Salamone and Correa, 2012), that has been associated with social dominance (Davis et al., 2009; Kunkel and Wang, 2018). However, we did not detect any differences in the time to push the opponent out of the tube between the genotypes during the winning trials in the tube test, we can exclude that *a priori* differences in motivation lead to the herein detected changes in social dominance.

Dopamine is well-known for modulating appetitive and aversive motivational processes through binding to D1 or D2 receptors in the NAc (Robbins and Everitt, 2007; Salamone et al., 2015; Salamone and Correa, 2012). A dyadic competition engages D1-containing neurons in the NAc of rats (Hollis et al., 2015), while antagonizing D1 receptors in this brain region abolishes the animals' chances to become dominant (van der Kooij et al., 2018). These data strongly support a key role of dopamine signaling, more specifically within the NAc in the establishment of social hierarchies. Interestingly, glucocorticoids can stimulate dopamine release in the NAc (Barrot et al., 2000; Piazza et al., 1996; Piazza et al., 1996). This effect is probably not due to a direct action of glucocorticoids on dopamine neurons in the ventral tegmental area (VTA) but may indirectly act through a feedback from the NAc to the VTA. Indeed, whereas GR gene inactivation has no effect on dopamine neurons spontaneous firing within the VTA, GR gene inactivation in the entire brain or in dopaminoceptive neurons (GR^{D1Cre} mice) decrease dopamine neurons spontaneous firing (Ambroggi et al., 2009; Parnaudeau et al., 2014). In addition, while repeated social defeat is known to induce a long-term increase of dopaminergic activity, that effect is abolished in GR^{D1Cre} mice (Barik et al., 2013). It is of note that in this mouse model, D1-expressing but also a large part of D2-expressing neurons is recombined (Barik et al., 2013). This precludes a direct comparison with the pharmacological approach using D1 receptors and will require to refine molecular genetics approaches to specifically pinpoint the role of the different NAc cell populations in the observed phenotype. The combination of our results in this model along with the ones obtained in the NAc GR knockdown experiment in rat however strengthen the idea that GR within this brain region might modulate social hierarchy through an effect on the mesolimbic dopamine pathway activity.

Another interesting observation in our study was that the knockdown of GR in the NAc not only promoted dominance, but also decreased anxiety levels in these animals compared to their respective controls. We previously highlighted a link between anxiety and social dominance, with low anxious animals exhibiting greater offensive behaviors than high anxious counterparts (Hollis et al., 2015) similar to those levels achieved by pharmacological reduction of anxiety using diazepam (van der Kooij

et al., 2018). Interestingly, low anxiety has also been previously linked with higher intermale levels of aggression in a rat model selected for anxiety extremes (Veenema et al., 2007). While the social competition described here does not equate to aggression, our findings are in line with the literature highlighting anxiety as a crucial factor for social competition and social status in both rodents (Hollis et al., 2015; Larrieu et al., 2017; van der Kooij et al., 2018) and humans (Gilbert et al., 2009; Goette et al., 2015).

The fact that we detected lower GR mRNA levels in submissive-prone rats compared to dominant-prone rats, but GR downregulation in the NAc led to reduced anxiety and higher social rank, might appear conflicting at a first glance. However, we should mention that in the first experiment, rats were selected according to their trait anxiety. In the GR downregulation experiment, we counterbalanced the anxiety levels in GR-KD and SCR rats, which led to equal chances to win or lose the social encounter, at least before the viral infusion. Interestingly, previous work has shown that animals bred for high anxiety exhibited HPA axis hyperactivity at baseline and after exposure to stress (Landgraf et al., 1999). Consistent with this finding, we detected greater levels of corticosterone in high anxious, subP rats than in low anxious, domP animals at basal state. The reduced GR expression in the NAc of high anxious, subP rats might therefore respond to a down-regulation induced by their higher glucocorticoid levels, suggesting that the observed impact on social dominance may depend on glucocorticoid signaling rather than on GR expression itself. Another support for the hypothesis of the importance of the balance of corticosterone and its receptor is brought by the significant lower expression in the NAc of a downstream target, i.e. the FKBP5 gene, in subP rats compared to domP animals and its positive correlation with GR gene expression (Figure S2). SGK-1 gene expression did not differ between groups. Interestingly, corticosterone levels were similar between GR-KD and SCR rats and no significant difference in either the FKBP5 or SGK-1 gene expression or their correlations with GR gene expression was found in those animals.

In conclusion, we highlight GR in the NAc and dopaminoceptive neurons, respectively, as important players in the establishment of social rank in rodents. In order to further strengthening the specific

role of accumbal GR on these processes, future studies should genetically target GR expression in dopaminoceptive neurons in the NAc. To date, our study represents a critical step forward on the understanding of the neurobiological mechanisms that regulate the attainment of social rank and may open new prospects for the advancement of preventive therapeutic approaches to psychiatric disorders characterized by aberrant social traits.

Author contributions

Conceived and designed the experiments: AP, FT and CS. Performed the experiments: AP, MW, AB, IZ, FH, TL, DB, and SP. Provided technical support: JG and OZ. Analyzed the data: AP, AB, IZ, FH and SP. Wrote the manuscript: AP, MW, SP, FT and CS. Obtained funding and supervised the study: FT and CS. All authors have approved the final version of the manuscript.

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Conflict of interest

We declare no conflict of interest.

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Appendix A. Supplementary data

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