Social status influences normal and pathological behaviors in mice, a role for dopamine and stress signaling

Dorian Battivelli[1], Cécile Vernochet[1], Claire Nguyen[2], Soumee Bhattacharya[1], Abdallah Zayed[1], Aura Carole Meirsman[1], Sarah Messaoudene[1], Alexandre Fieggen[1], Jean-Pol Tassin[1], Fabio Marti[2], Philippe Faure[2], Sébastien Parnaudeau[1]*, François Tronche[1]*

[1] Gene Regulation and Adaptive Behaviors group, Neuroscience Paris Seine, UMR8246, Centre National de la Recherche Scientifique (CNRS), INSERM, Institut de Biologie Paris Seine, Sorbonne Université (SU), 75005, Paris, France.

[2] Neurophysiology and Behavior group, Neuroscience Paris Seine, UMR8246, Centre National de la Recherche Scientifique (CNRS), INSERM, Institut de Biologie Paris Seine, Sorbonne Université (SU), 75005, Paris, France.

* co-last authors.

Correspondence to François Tronche (francois.tronche@upmc.fr) and Sébastien Parnaudeau (sebastien.parnaudeau@upmc.fr)

Keywords: social ranking, behavior, dopamine, depression, addiction, glucocorticoid receptor.

Summary

The rules leading to the emergence of a social organization and the role of social ranking on normal and pathological behaviors remain elusive. Here we show that groups of four genetically identical male mice rapidly form enduring social ranking determined by precedence test and the sharing of beneficial resources. Highest ranked individuals are more anxious, more social and display increased spatial working memory. Whereas differences in anxiety between individuals appear after rank attainment, the higher sociability of top-ranked mice preexist. These behavioral differences correlate with physiological change. The highest ranked mice display indeed lower bursting activity of VTA dopamine neurons. The same animals are less responsive to preclinical models of stress behavioral disorders involving changes of dopamine system. They display lower locomotor sensitization to cocaine and are more resilient to repeated social defeat. The ablation of stress-elicited glucocorticoid receptor gene in dopaminoceptive neurons that affects the same pathological models, upwards the ranking status of mutant individuals. Altogether, these results support a role for social ranking in patterning interindividual VTA dopaminergic activity, behavioral responses and susceptibility to stress-related psychopathologies.

Introduction

Social organization is readily observable across vertebrate species and can result in the establishment of a social hierarchy that may minimize energy costs due to direct competitions for resources among congeners (Tinbergen, 1939; Francis, 1984). At the group level this may improve adaptation to the environmental demands. At the individual level, it exposes different congeners to distinct experiences and participates to the generation of individuality, the consistency of an animal in his responses to environmental and social challenges such as how to find food, deal with predators, or compete with conspecifics, that distinguishes it from others (Bergmüller and 2010, Lathe 2004).

Mice are social vertebrates, living in hierarchical structures of 4 to 12 adult members (Berry 1992, Beery 2015) that share territorial defense and exhibit several behaviors (e.g. physical exploration, vocal communication, aggression, social recognition, imitation, empathy) that characterize sociability. The social rank of individuals can be determined based on observations of antagonistic interactions, territorial marking, access to limited resources and by precedence behaviors (Zhou *et al.*, 2018). The driving forces underlying the emergence of social organization remain largely unknown. These include genetic factors, however, the fact that social hierarchy is observed within groups of genetically identical congeners suggests that environmental factors are in play. Among these, stress response and more specifically

glucocorticoids release has been suspected to influence social dominance in a variety of species, although a clear link is not established (Sapolski 2004, Creel *et al.* 2012).

Hierarchy establishment involves iterative pairwise interactions that have consequences on the behavioral fate of each individual (Cordero and Sandi, 2007; Timmer and Sandi, 2010). Indeed, specific behavioral patterns emerge in genetically identical mice raised in semi-naturalistic environments (Freund *et al.* 2013; Hager *et al.* 2014; Torquet *et al.* 2018), and differences in behavioral traits have been attributed to social ranking in smaller colonies (Wang *et al.* 2011; Larrieu *et al.* 2017). Whether such individual differences preexist the formation of the social group is unclear, and the physiological mechanisms implicated in hierarchical segregation remain elusive. Beyond understanding the principles of interindividual behavioral diversity, these questions are also relevant in a psychopathological context since social status is recognized as a vulnerability risk factor for psychiatric diseases including mood disorders and addiction (Kessler *et al.* 1994, Wilkinson, 1999, Lorant 2003, Singh-Manoux *et al.* 2005)

The mesocorticolimbic system that encompasses the prefrontal cortex (PFC), the nucleus accumbens (NAcc) and their dopaminergic input from the ventral tegmental area (VTA) could participate to the emergence of social hierarchy and behavioral diversity. This brain system modulates a broad spectrum of behaviors, including motivation and decision-making involved in social context (Gunaydin *et al.*, 2014). VTA dopamine neurons activity conditions social avoidance following social defeats (Chaudhury *et al.*, 2013, Barik *et al.* 2013). The interaction between stress-evoked release of glucocorticoids and dopamine system is critical for this effect and relies on the activation of glucocorticoid receptors (GR) present in dopaminoceptive neurons (Barik *et al.* 2013). Several structures receiving dopaminergic inputs have been recently associated with the emergence of social ranking. Modulating the synaptic efficacy in medial PFC neurons causes individual bidirectional shifts within social rank (Wang *et al.* 2011). Also, lower mitochondrial activity within the nucleus accumbens is associated to lower social ranking in both rats and mice (Hollis *et al.* 2015; Larrieu *et al.* 2017).

In this study, we examined the divergence of normal and pathological behaviors with social status in colonies of four genetically identical mice. We investigated whether differences were preexisting rank specific behavioral traits. Finally, we provide evidence for a potential implication of mesocorticolimbic brain system and stress response signaling in these processes.

Results

Social rank within tetrads is stable over long periods

We formed colonies of four weight-matched adult C57BL/6J male mice (tetrads), previously unknown to each other, and analyzed the individuals social rank, two to three weeks later. We first used a precedence test based on encounters within a plastic tube between each possible congener pairs among the tetrad that allows to identify lower ranked individuals as they come out of the tube walking backward (Fig. 1A, Wang *et al.* 2011). Briefly, three times a day, the six possible pairwise combinations of individuals from a tetrad were tested three times, and the one with the highest number of forward exits considered higher ranked. We tested each tetrad daily, for at least six days, until the highest and lowest ranks (rank 1 –R1- and rank 4 –R4-, respectively) were stable over 3 consecutive days. Among 60 tetrads presented in Fig. 1B, the stability criterion was reached faster for the extreme ranks (1 and 4). Half of R1 and R4 mice were already having a stable status on days 3 and 5, respectively. All of them were stables after 12 days whereas a quarter of ranks 2 and 3 were not. As observed by Wang *et al.* (2011), the rank of individuals conditioned the duration of contests. Confrontations between R1 and rank 2 (R2) individuals lasting for around 18 s whereas confrontations involving R4 lasted less than 9 s (Fig. 1C).

Once established, social ranking was stable over long period. Fig. 1D pictures social destinies of individuals from twelve tetrads repeatedly assessed through 5 sessions, during 17 weeks (Fig. 1D). This is particularly true for R4 individuals (Fig. 1D, red lines) since, 17 weeks later, 11 out of 12 mice remained at the lowest rank. Among initially highest ranked individuals, 10 and 7, out of 12 kept the same ranking, 14 and 17 weeks later, respectively (Fig. 1D, green lines). One progressively decreased ranking, to end in rank 4, one ended in rank 3 and three in rank 2. Animals with initial intermediate ranks displayed the highest exchanged rankings but 19 out of 24 still ended by attaining an intermediate rank (Fig. 1D, blue, orange and black lines). During this period of time, mice were regularly weighted, and no correlation between social rank and weight evolution was found (data not shown).

To validate the tube test as a ranking test we quantified other expressions of social dominance, such as territorial urine marking and warm-plate occupancy in a cold environment. Urine marking patterns were collected on absorbent paper from a box occupied by R1 and R4 individuals, separated by a transparent and perforated wall, during 2 hours, and visualized under U.V. light (Fig. 1E). 17 out of 23 top-ranked individuals in the tube-test showed also dominant urine marking patterns, in either the number of marks or their cumulated area, when compared with lower ranked congeners. Similarly, higher ranked individuals in the tube-test deployed significantly longer occupancy of a small warm spot within a cold cage during the 20 min of the test (Fig. 1E) compared to their three others cage-mates.

Social rank correlates with behavioral differences.

We compared behaviors between identified R1 and R4 individuals. Locomotor activity of both ranks was similar when measured in an open-field (Fig. S1). This was confirmed when activity was measured in circular corridors (see below). Despair, a depression-like behavior was measured by quantifying immobility and escape behaviors in the forced-swim test for two consecutive days. As expected, we observed a general increase in immobility between the first and the second days, particularly marked for the first minutes of the test (Fig. 2A upper panels). Individuals from both ranks displayed similar immobility and escape behaviors.

We then quantified anxiety-like behavior in two approach-avoidance conflict tests based on the mouse innate avoidance of open and lit spaces. Among genetically identical C57BL/6 mice, R1 individuals present more anxiety-like behaviors than R4 ones. They spent significantly less time in the open section of an elevated O-maze (Fig. 2B, left) and in the lit compartment of a dark-light box (Fig. 2B, right). Furthermore, R1 mice displayed increased sociability when compared to R4 ones. As expected, when testing 115 C57BL/6 mice for their level of interaction with an unfamiliar C57BL/6 mouse placed within a transparent plastic box *vs* an empty box, they display a marked preference for a social stimulus (Fig. 2C, social preference, black bars). However, stratification of these individuals taking into account their social rank in home-cage shows that only the highest ranked individuals, and not the lowest ones displayed social preference (Fig. 2C, social preference, right panel, green and red bars, respectively). Social rank does not however affect social memory or social novelty. Mice have a natural preference for interacting with an unfamiliar conspecific *vs* a familiar one. We observed indeed (Fig. 2C, social memory, black bars) such a preference for the 115 C57BL/6 individuals but is was similar for both R1 and R4 males (Fig. 2C, social memory, green and red bars, respectively).

We then addressed whether social ranking could also affect cognitive abilities. We studied spatial working memory in a non-match-to-sample T-maze task (Fig. 2D, upper panel). In this task, mice are placed within a T-Maze and can access a reward placed into the unique open arm (forced phase). They are required to retain a memory trace of a recently sampled maze location during a delay period (delay phase) and then prompted to select the opposite location in order to receive a reward (choice phase). Each mouse was tested 10 times a day, and the learning criterion was defined as a minimum of 7 correct choices for 3 consecutive days. Although both groups of C57BL/6 mice learned the task, R1 individuals did it significantly faster (Fig. 2D, lower left panel).

Differences in sociability but not anxiety-like behaviors pre-exist to social rank establishment

The behavioral differences between ranks could emerge from initially similar mice as a consequence of social adaptation within members of a tetrad, initially similar. Alternatively, these differences could pre-exist before their gathering, and shape individual social ranking trajectories. To address this question, we compared behavior of individuals before grouping them in tetrads, and after the formation of the social colony. O. R1 and R4 did not display differences in despair-like behavior, as thus expected we did not observe either differences between future R1 and R4 (Fig. 3A). Anxiety-like behaviors were markedly enhanced for highest ranked individuals compared to lowest ones. This behavioral difference seems to emerge from social organization since no differences were observed, neither in elevated O-maze, nor in dark-light tests between future R1 and R4 individuals. These two groups and the future ranks 2 and 3 were similar with the time spent in open arms and in the lit compartment, and presented similar individual dispersions (Fig.3 B). The origins of differences in sociability seem to be drastically different. Future R1 mice have already a marked appetence for social interactions before social life in tetrads, similar to that observed once the tetrad is formed (Fig. 3C, green bars, left panel), whereas future R4 have not. Interestingly, intermediate ranks have an intermediate phenotype with a significant but lower preference. Of note, the animals presented in Fig.3 are a subgroup of those presented in figure 2. When examined separately they showed significant differences in anxiety-like and social behaviors upon social ranking establishment.

Rank 1 and 4 individuals present differences in dopaminergic mesocorticolimbic activity

We previously reported that social aversion, induced by repeated social defeat, was engaging changes in mesocorticolimbic dopaminergic system activity modulated by stress elicited glucocorticoid hormones release. We therefore investigated whether differences could exist in the activity of dopamine cells from the VTA between R1 and R4 individuals. We performed extracellular single-unit recordings in anesthetized mice and observed significant differences between these two ranks from different social rank (Fig. 4A, left panel). The analysis of 186 and 157 neurons from 10 R1 and 10 R4 mice, respectively revealed that whereas the frequency of firing was similar in both ranks (Fig. 4A, left graph), the percentage of spikes within bursts (SWB) was significantly lower in R1 (Fig. 4A, right graph). VTA dopamine neurons project to the PFC and the NAcc. To assess dopamine release, we measured in these structures the amounts of dopamine (DA) and 3,4-Dihydroxyphenylacetic acid (DOPAC), a metabolite produced following dopamine reuptake, and calculated the DOPAC/DA ratio that give an index of the release (Fig. 3B). In the NAcc, we noted a trend towards a decreased DA release in R1 individuals when compared to R4 ones, although the difference did not reach significance (Fig. 4B). No difference was observed in the caudate putamen (CPu), mostly

innervated by dopamine cells located in the Substantia Nigra pars compacta. In striking contrasts, in the PFC, R1 individuals displayed a marked increase of DA release. Overall, we showed a DA release characterized by a stronger cortical-subcortical hierarchy in R1 mice compared to R4 ones.

Social rank conditions sensitivity to some preclinical models of psychopathologies

Differences in dopamine circuits activity have been associated with propensity to develop several psychopathologies including addiction and depression. In mouse models, the mesocorticolimbic system modulates behavioral responses to psychostimulants as well as the appearance of social aversion following repeated defeats. We therefore investigated whether highest and lowest ranked individuals within tetrads would respond distinctly to these preclinical models. We studied locomotor sensitization to cocaine in both ranks. This gradual and enduring facilitation of locomotor activity promoted by repeated cocaine exposure is believed to reflect the reinforcing effects of abused drugs (Robinson and Berridge, 2000). R1 and R4 mice were habituated to the display for three days, receiving a saline injection on the last two days. For the five following days animals received daily injections of cocaine (10 $mg.kg^{-1}$). This incremented from day to day their locomotion during the following hour (Fig. 5A, left graphs, compare the time course after saline injection and days 1, 2 and 5). After 6 days of withdrawal, we performed a last injection (challenge day). Whereas repeated cocaine treatment induced a marked locomotor sensitization in both social ranks, lowest ranked individuals had a significantly higher locomotor sensitization and this difference was maintained on the challenge day (Fig. 5A, right graph).

Repeated social defeats induce enduring increase of VTA dopamine neurons activity and social aversion, considered a model of depression, this behavioral change is reversible by antidepressant treatments (Berton *et al.* 2006). When performed with C57/BL6 mice this protocol leads to high interindividual differences in the appearance of social aversion, with usually 60% of susceptible individuals whereas the other are resilient, a difference in part explained by differences in VTA dopamine neurons. Highest and lowest ranked mice from eight tetrads were subjected for 10 days to social defeats (Fig. 5B, upper right graph). We analyzed their interaction with an empty plastic box *vs* a box containing an unfamiliar CD1 congener, before and after social defeat. After social defeats, 7 mice, out of the 16 C57B/L6, developed a social aversion. Among them only one was a R1. In other words, majority of R1 individuals (87,5%) were resilient whereas only 25% of R4 ones were (Fig. 5B, lower graphs).

GR gene inactivation in dopaminoceptive neurons facilitates higher social ranking

Some of the phenotypes observed in R1 mice are reminiscent of that of GR^{D1aCre} mice, that are deprived of glucocorticoid receptor gene within dopaminoceptive neurons. These mice,

compared to control littermates, have a reduced VTA dopamine neurons activity, weaker behavioral responses to cocaine, including locomotor sensitization, and are more resilient following repeated social defeats. We wonder whether such a change in dopaminergic activity would differently shape the social destiny of mutant mice within a tetrad colony. To address this question, we grouped one GR^{D1aCre} mice with three unfamiliar control individuals (Fig6., left graph) and assessed their social rank in tube-test 3 weeks later. GR^{D1aCre} ended on the highest rank within 5 tetrads out of 7, in one it was ranked second and in one fourth (Fig5., middle graph). To investigate whether HPA-axes might differ depending on social ranks, we weighted the adrenal glands and the thymus of R1 and R4 and did not observe differences (Fig S2).

Discussion

Within a few days, genetically identical mice living in small groups of four individuals establish a social organization that can be observed during differential precedence in displacements, measured in tube-test, and differential access to resources, such as a warm spot in a cold environment, as was previously reported by several groups (Wang 2011, Larrieu 2017). The attained social rank is stable over month periods, with limited exchanges between ranks within a tetrad. These exchanges are almost absent for the lowest ranked individuals and rarely observed for the highest ones. They are frequent for the two intermediate ranks that also take a longer time to stabilize during the first tube-test session. Behavioral analyses usually present an important interindividual variability. Social ranking might be in part responsible for these variations. We showed, in agreement with others, that highest ranked mice exhibit indeed higher anxiety-like behaviors, and increased social interactions. The increased anxiety level has been reported by Larrieu *et al.* 2017, using the same ranking approach that we did. A recent report, did not however observe this correlation. This discrepancy may rely on the limited number of animal tested, or on the approach chosen to identify ranking, with sparser tube tests, performed once a week for three weeks (Varholick *et al.* 2018). Few other studies using other criteria to identify dominant individuals, such as aggressiveness, have been carried out leading–to conflicting results. As such, Hvaliki 1989 reported no differences whereas Ferrara *et al.* 1998 saw a reduced anxiety for dominant individuals. Similarly, the high dispersion of individual interaction time with a congener during sociability tests in isogenic mice can also be in part explained by their social rank. Highest ranked ones are indeed more sociable, in agreement with Kunkel *et al.* (2018). This association of high anxiety and high sociability is surprising since in both human and rodent, low anxiety is usually paired with increased sociability (Allsop *et al.*, 2014, Beery and Kaufer 2015). For instance, oxytocin

enhances social function and has well-known anxiolytic properties (Insel *et al.* 2010). In the same line, optogenetic stimulation of basolateral amygdala to ventral hippocampus circuit facilitates anxiety and impairs social interaction (Felix-Ortiz *et al.* 2011, Felix-Ortiz and Tye 2014). This associative rule is nevertheless not systematic. Similar to our observation in top ranked individuals, vasopressin promotes social behavior and is also anxiogenic (Bielsky *et al.* 2004).

A central but poorly explored question is whether the emergence of social ranks precedes the appearance of specific individual behavioral traits, or whether preexisting individual differences channel the social status trajectory of an individual. Our study indicates that both situations occur. The anxiety of highest ranked animals clearly emerged following social life since no differences existed prior to rank establishment. A similar observation was made in outbred Swiss mice, housed in dyads and ranked upon their aggressiveness (Hilakivi-Clarke 1992). On the contrary, in rats, a study showed that a high level of anxiety is a predisposing factor for social submission (Hollis, 2015). In our study, while the increased anxiety seems to be the consequence of social ranking, the difference in sociability clearly preexists to the formation of the ranks within the colonies. The origin of this individual difference in behavior most likely arise due to previous breeding and social housing conditions. It could have emerged in the first colony in which these animals were grouped. It could also occur from differences that appeared early, before weaning, since a study suggested that maternal care could shape adult social behavior (Starr-Philipps and Berry 2013).

Several studies point at the mesocorticolimbic system as a potential substrate for social ranking. In the NAcc, low mitochondrial activity has been causally linked with lower rank in dyadic contests (Hollis *et al.* 2015). Also, increased activity and higher strength of excitatory inputs to the PFC layer V has been linked with higher social ranking (Wang *et al.* 2011). Our study shows that VTA dopamine neurons exhibit differential activity depending on the rank, with a marked reduction of the bursting activity in R1 individuals. Several studies suggest a role for dopamine in social ranking from insects to mammal with different correlations made (Yamagushi *et al.* 2015). In ants, brain dopamine concentration is higher in socially dominant individuals (Penick *et al.* 2014, Okada *et al.* 2015). In birds and lizards, increased levels of dopamine in striatal structure have been observed in higher ranked individuals (McIntyre and Chew 1983, Korzan *et al.* 2006). In line with our results, reduced levels of dopamine have been shown in the NAcc of dominant rats (Jupp *et al.* 2016). However, optogenetic stimulation of VTA dopamine neurons has been recently shown to favor dominant behavior for competitive access to reward, which could rather reflect the role of this brain region in reward processing (Lozano-Montes *et al.*, 2019).

Genetic evidences also sustain a link between dopaminergic neurotransmission and social status. Dopamine transporter gene is essential for sensing dopamine release and

dopaminergic neurotransmission. Its inactivation in mice disorganizes social colonies (Rodriguiz *et al.* 2004), and genetic variants of the DAT gene are associated with social dominance in macaques (Miller-Butterworth *et al.* 2008). Imaging studies in human and non-human primates showed an enhanced availability of the striatal D2 receptor for individuals with dominant status. This could result from either higher level of D2 receptors or lower dopamine release (Nader *et al.* 2012, Cervenka *et al.* 2010, Martinez *et al.* 2010). Neuropharmacological approaches also suggest a role for dopamine signaling in social ranking but the differences in strategies deployed (e.g. systemic *vs* local striatal injections in the NAcc) do not allow clear interpretation. Systemic administration of D2 receptor antagonist reduced social dominance in both mice and monkeys (Yamaguchi *et al.* 2017a) whereas local injection into the NAcc of an agonist did not have an effect in rats (Van der Kooij 2018). Similar experiments with a D1 receptor antagonist facilitated or did not modify social dominance in mice and monkeys (Yamaguchi *et al.* 2017b) whereas local injection into the NAcc of an agonist increased dominance in rats (Van der Kooij 2018). Interestingly, changes in VTA dopamine cells activity is observed during the emergence of behavioral categories occurring within groups of dozens of mice living in complex semi-naturalistic environments (Torquet *et al.*, 2018). It will be interesting to study social ranking between these categories using precedence test such as the tube test. The decreased firing in R1 mice was associated with a trend of decreased dopamine release in the NAcc as measured by the ratio DOPAC/DOPA, but also more surprisingly with an increased release in the PFC which could explain their enhanced working memory ability. This apparent discrepancy between our electrophysiology data and the increased cortical release of dopamine is likely due to the fact that only a minority of the dopamine cells in the VTA project to the PFC (Björklund and Lindvall, 1984).

Dysregulation of the mesocorticolimbic system is a key feature of several stress-related behavioral psychopathologies, including addiction and depression that develop with a high interindividual variation that is not fully understood (Robinson and Nestler, 2011; Russo *et al.*, 2012). The differences of cortical/subcortical dopaminergic balance between the higher and lower ranked individual we reported may provide a physiological ground underlying differential vulnerability to psychopathology-like phenotypes. Indeed, the reduction in locomotor sensitization to cocaine in highest ranked individuals is coherent with the reduction of VTA bursting activity in these individuals (Runegaard, 2018). Repeated social defeat in mice has been intensively used as a preclinical model of depression. In a subset of animals, so-called susceptible, this chronic stress induces enduring anxiety and social avoidance that depends on enduring increase of VTA dopamine activity (Cao *et al.* 2010). Optogenetic stimulation of VTA neurons projecting to the NAc induces a susceptible phenotype whereas optogenetic inhibition induces resilience (Chaudhurry 2013). We demonstrated that lowest ranked mice, with higher VTA dopamine tone are more likely to develop social aversion following ten days

of repeated defeats. Two other studies made observations that differ from ours on the consequences of repeated social defeat depending on social rank. Lehmann et al (2013) did not observe a correlation whereas Larrieu *et al.* (2017) observed the opposite (*i.e.* resilience for lower ranked individuals). Differences may reside in the intensity of the defeats to which individual were exposed, the lower number of R1 and R4 tested (8 here *vs* 4) and the fact that pooled data from ranks 1 and 2 were compared to that of ranks 3 and 4. Furthermore, our experiment was performed on tetrads established for 5 months that have been tested regularly to ensure their stability over time. This repeated solicitation of animals in the context of chronic social competitions may have reinforced the phenotypes of each rank.

Studies in human and animals suggested the existence of a correlation between social rank and differences in stress hormones Elevated circulating glucocorticoids are usually associated with subordinate status in non-mammals, and mammals including rodents and primates, although conflicting results have been reported (Sapolsky 2004, Sapolsky 2005, Creel *et al.* 2012, Cavigelli and Caruso 2015). In human, the common perception that dominant individuals may have higher glucocorticoid levels has been challenged. Higher socio-economic status (SES) has been linked to lower evening glucocorticoid levels (Cohen *et al.* 2006). Studies in military leaders, as well as in influential individuals from a bolivian forager-farmer population, different for individuals with higher SES, showed lower glucocorticoid levels (Sherman *et al.* 2012, von Rueden *et al.* 2014). We studied the social fate of mice deprived of the glucocorticoid receptor gene in dopamine innervated neurons. This targeted mutation clearly promotes higher social ranking in our tetrads. This result is consistent with our recent observation made on mice raised by two (Papilloud *et al.* 2019). Interestingly, we also showed that these mice exhibit a lower VTA dopamine cells bursting activity (Ambroggi *et al.* 2009), a decreased sensitization to cocaine (Barik *et al.* 2010) and a shift toward resiliency following repeated social defeat (Barik *et al.* 2013). These phenotypes are strikingly similar to that of R1 individuals suggesting that stress response and its impact on dopamine pathway might play a principle organizational role in shaping the behavioral trajectories leading to the establishment of social ranking.

Material and method

Animals

C57BL/6JRj, 129/SvEv, and CD1 male mice were purchased from Janvier (Le Genest-Saint-Isle, France) and housed under standard conditions, at 22°C, 55% to 65% humidity, with a 12-hour light/dark cycle (7 am/7 pm) and free access to water and a rodent diet. *Nr3c1* (*GR*) gene inactivation was selectively targeted in dopaminoceptive neurons ($Nr3c1^{loxP/loxP}$;(Tg)D1aCre - Lemberger *et al.*, 2007, here after designed GR^{D1aCre}), as described in Ambroggi *et al.* (2009).

Experimental animals were obtained by mating $Nr3c1^{loxP/loxP}$ females with $Nr3c1^{loxP/loxP}$;Tg:D1aCre males. Half of the progeny were mutant animals the other half were control littermates. When required, thymus and adrenal glands of animals were dissected and weighted after fat tissue removal under a binocular loupe. Experiments were performed in accordance with French regulation (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.

Constitution of tetrads

Six weeks-old male mice were weighted upon arrival and were then grouped by four (tetrads) gathering mice of similar weights. When behavioral testing was performed before the constitution of the tetrads, mice were singly housed for one week. Mice were regularly weighted. For tetrads including GR^{D1aCre} mutant mice, animals were genotyped at 4 weeks of age. Tetrads were formed with animals unfamiliar to each other issued from different litters, grouping one mutant with four control mice($GR^{loxP/loxP}$) of the same age.

Social rank identification

Tube-test. Mice gathered by groups of four individuals for two to four weeks were first trained to move forward a transparent Plexiglas tube (diameter, 2.5 cm; length, 30 cm) for 2 consecutive days, performing 8 trials the first day and 4 the second one. Each individual alternatively entered the tube from right and left extremities and was let for a maximum of 30 s to exit the tube at the opposite end. After 30 s if still present within the tube, the mouse was gently pushed out. The diameter of the tube allowed passing one individual but and did not permit it to reverse direction. During the following days, social ranks were assessed daily through the six possible pairwise confrontations in the tube, performing for each a trial composed of 3 confrontations. Two mice were simultaneously introduced within the tube from the 2 opposite ends taking care that they met in the middle of the tube. The first mouse to exit the tube was designed as the looser of the contest. The individual that won at least 2 confrontations was ranked higher. Mice were classified from rank 1 (3 wins) to rank 4 (no win), the lowest. Contests exceeding 2,5 min were stopped and immediately repeated. After each trial, the tube was cleaned with 20% ethanol and dried. Among 84 tetrads analyzed, we always observed a non-ambiguous ranking. The order of confrontations was randomized day after day using a round-robin design. Social ranks were initially assessed during a minimum period of 6 days and considered stable if both ranks 1 and 4 were stable for the last three days. Tetrads that did not reach this criterion were analyzed further, until reaching a three days stability for ranks 1 and 4. Among 60 tetrads, all reached stability within 12 days. Social rank was repeatedly analyzed every three to four weeks for a minimum of three consecutive days.

Territory urine marking assay. R1 and R4 mice from a tetrad were placed in an empty PVC box (42 x 42 x 15), separated by a central transparent perforated Plexiglas divider and were let free to explore and mark their own territory for 2 hrs. One piece of absorbent paper (Whatmann), partially covered by fresh sawdust was set in the bottom of each compartment to collect urine deposited by mice during the session. Absorbent paper was then pictured under UV light (312 nm). Both the number of urine marks and the total area of urine marks were quantified.

Warm-plate assay. Tetrads were placed in a transparent plastic cage (35 x 20, 18) without litter, placed on ice (bottom cage temperature 4 °C). 20 min later, a warm plate (11 x 9 cm, 28-30 °C) was introduced on the floor of the cage, at a corner. Mice activity was recorded for 20 min, and warm plate occupancy, by each individual, scored by a blind experimenter.

Spontaneous locomotor activity in open field

Mice were placed in a corner of a squared PVC white box (42x42 cm, 15 cm depth), and let free to explore for 10 min, under 50 lux. A video camera system placed above enabled the automatic quantification of locomotor activity (Noldus Ethovision 11.0 XT).

Anxiety-like behavior

Dark-Light box. The dark-ligth box apparatus consisted of a plastic rectangular box (45x20 cm, 25 cm high) divided into a white compartment (30 cm, open) and a black compartment (15 cm, covered with a removable lid), that communicate through a central door (5 x 5 cm). Animals were initially placed into the black compartment, and exploration recorded for 10 min, under 30 lux. The time spent in each compartment was blindly scored by two experimenters.

Elevated zero-maze. The maze consisted of a circular path (width 5,5 cm, outer diameter 56 cm) elevated 30 cm above the floor and made of black PVC. It was divided in four sections of equal lengths, two opposite bordered with bilateral black plastic walls (15.5 cm high) and two open ones. Mice were positioned at one extremity of a closed section, the head directed inward, under 50 lux in the open sections and 10 lux in the closed one. Their exploration was recorded for 10 min and the time spent into closed and open sections was blindly scored by two experimenters. A mouse was considered to be in a section when the 4 paws were introduced.

Despair Forced swim test

Glass cylinders (40 cm tall, 12 cm diameter) were filled with tepid water (23°C) until reaching a depth of 10 cm. Mice, placed on a large spoon, were gently introduced into cylinders and videotaped for 6 min. Cumulative length of time of immobility, balance and escape movements were blindly scored. Escape behavior was defined as movements involving the 4 paws of the

animal beating against the wall of the cylinder mimicking a climbing-like behavior. Balance movements refer to brief movements involving mainly only the 2 posterior paws of the animal and aiming to displace in water without trying to climb up the cylinder's wall. Mice were considered immobile when floating passively, doing neither escape nor balance movements. The experiment was repeated 24 h later when planed in the experimental design.

Sociability, three-chambers test

Sociability was measured under 50 lux in a rectangular box containing three chambers (30×20, 15 cm high for each compartment) with removable doors (5×5 cm) at the center of each partition. In the opposite sides of the 2 lateral compartments, 2 clear perforated plastic boxes (10x7 cm, 7 cm high) were placed. One contained an unfamiliar adult male mouse (C57BL/6J), the other was left empty. During habituation phase (5 min), the challenged individual was placed in closed central compartment. Doors were then opened and the mouse free to explore the display for 5 min. The sessions were registered, and the close interaction time with the empty box and with the box containing an unknown congener were blindly scored. The interaction time was defined as the periods during which the animal was oriented with the head towards the box, and in direct contact with it. To measure the preference for social novelty, and social memory, the mouse was let, closed, in the chamber containing the social cue for 5 min. It was then placed again in the central chamber, free to investigate the three compartments. The time length spent in close interaction with boxes containing, either a familiar mouse, previously encountered, or an unfamiliar one was scored, during 5 min session.

Non-matching to sample T-maze task

The test was performed as previously described (Sigurdsson *et al.*, 2010). Briefly, mice underwent for 3 days a moderate food reduction (2 g/mouse/day), taking care not to go below 85% of their initial weight. Animals were then trained on a spatial working memory task (non-match-to-sample task) in a T-maze (61 cm large x 51 cm width x 15 cm high, with a path 11 cm large). Mice were habituated to the maze for two days during which they had 15 minutes to collect food pellets (20mg dustless sugar pellets, Bioserv). The next three days, mice had to complete 4 forced runs each day, during which one of the two arms were alternatively closed in order to habituate to the guillotine doors (Fig. 2D). Mice were then daily tested on 10 trials per day. Each trial consisting of two runs, a forced run and a choice run. At the beginning of the trial both arms are baited. In the forced run the right or left arm is randomly chosen to be opened, while the other arm is closed. At the beginning of the forced run, the mouse was placed at end of the longest T-maze arm. After running down this arm, it could enter into the open goal arm and have access to a food reward. Once the mouse reached back the starting

arm, it was blocked by a door at its end for a delay of 6 s. Then started the choice run. During it, the mouse ran down the center arm, where it had to choose between the two open goal arms. To obtain a reward, animals were required to enter the non-visited arm during the sample phase. This was scored as a correct choice. Animals were exposed to daily sessions of 10 trials, until they reached a criterion performance, defined as having a minimum of seven correct choices a day, for three consecutive days. The inter-trial time was 45 s.

Social defeat and interaction paradigms

Social defeat was performed as previously described (Barik *et al.* 2013). Six months old CD1 breeder male mice were screened for their aggressiveness. 6 months old individuals were subjected to 10 consecutive days of social defeat with new encounters. Each defeat consisted of 5 min physical interactions with a resident CD1 mouse, followed by a 24 h exposure to the CD1 in its home cage but separated by a perforated transparent plastic wall which allowed visual, auditory, and olfactory communication whilst preventing physical contact. Social interaction was first performed the day before the first social defeat (pre-defeat) and performed again 24 h after the last social defeat (post-defeat). Challenged mice were placed for 150 s in a plastic white open-field (42x42 cm, 30 cm high, 20 lux) containing an empty transparent and perforated plastic box. Mice were rapidly removed and an unfamiliar CD1 mouse was placed in the box, and the challenged mouse re-exposed to the open-field for 150 s Sessions were recorded and the times spent direct interaction with the box were manually quantified by an experimenter blind to conditions.

Locomotor sensitization to cocaine

Locomotor sensitization to cocaine was conducted on 3 months old R1 and R4 individuals. Mice were placed in a circular corridor (4.5-cm width, 17 cm-external diameter, 30-50 lux) crossed by four infrared captors (1.5 cm above the base), equally spaced (Imetronic, Pessac, France). The locomotor activity was automatically quantified by counting the quarters of turn travelled by the mouse that corresponded to the interruption of two successive beams (Cyclotron pour Imetronic, Bordeaux, France). Animals were habituated to the apparatus for 3 hours during 3 consecutive days and received a saline injection on days 2 and 3 (NaCl 0.9% saline solution, 10 ml/kg, *i.p.*). On the five following days, mice were placed in the apparatus for 90 min, then injected with cocaine hydrochloride (Sigma-Aldrich, 10mg/kg *i.p.*) and let inside 180 min after injection. Following 7 days of withdrawal, mice received a challenge injection of cocaine (10mg/kg *i.p*). At the end of each session, mice were placed back in their tetrads. Social ranks were tested at the end of the experiment, and only mice of R1 and R4 that did not change were considered for the analysis. The behavioral sensitization experiment has been carried out from 9 am to 13 pm.

In vivo electrophysiological recordings

3 to 5 months old R1 and R4 mice were anesthetized with chloral hydrate (8%), 400 mg/kg *i.p.* supplemented as required to maintain optimal anesthesia throughout the experiment, and positioned in a stereotaxic frame. A hole was drilled in the skull above midbrain dopaminergic nuclei (coordinates: 3.0 ± 1,5 mm posterior to bregma, 1 ± 1 mm [VTA] lateral to the midline, Watson and Paxinos 2010). Recording electrodes were pulled from borosilicate glass capillaries (with outer and inner diameters of 1.50 and 1.17 mm, respectively) with a Narishige electrode puller. The tips were broken under microscope control and filled with 0.5% sodium acetate. Electrodes had tip diameters of 1-2 µm and impedances of 20–50 MΩ. A reference electrode was placed in the subcutaneous tissue. The recording electrodes were lowered vertically through the hole with a micro drive. Electrical signals were amplified by a high-impedance amplifier and monitored with an oscilloscope and an audio monitor. The unit activity was digitized at 25 kHz and stored in Spike2 program. The electrophysiological characteristics of dopamine neurons were analyzed in the active cells encountered when systematically passing the microelectrode in a stereotaxically defined block of brain tissue including the VTA (1). Its margins ranged from -2,9 to -3,5 mm posterior to bregma (AP), 0,3 to 0,6 mm (ML) and -3.9 to -5 mm ventral (DV) (Grace and Bunney 1984). Sampling was initiated on the right side and then on the left side. Extracellular identification of dopamine neurons was based on their location as well as on the set of unique electrophysiological properties that distinguish dopamine from non-dopamine neurons *in vivo*: (i) a typical triphasic action potential with a marked negative deflection; (ii) a long duration (>2.0 ms); (iii) an action potential width from start to negative trough > 1.1 ms; (iv) a slow firing rate (<10 Hz and >1 Hz). Electrophysiological recordings were analyzed using the R software (https://www.r-project.org). dopamine cell firing was analyzed with respect to the average firing rate and the percentage of spikes within bursts (%SWB, number of spikes within burst divided by total number of spikes). Bursts were identified as discrete events consisting of a sequence of spikes such that: their onset is defined by two consecutive spikes within an interval <80 ms whenever and they terminate with an inter-spike interval >160 ms. Firing rate and %SWB were measured on successive windows of 60 s, with a 45 s overlapping period. Responses to nicotine are presented as the mean of percentage of firing frequency variation from the baseline +/- SEM. For statistical analysis, maximum of firing variation induced by nicotine occurring 180 s after the injection are compared to spontaneous variation from the baseline occurring 180 s just before the injection by non-parametric Mann-Whitney test.

Quantification of dopamine and DOPAC

Animals were decapitated and brains were rapidly dissected and frozen at -12°C on the stage of a Leitz-Wetzlar microtome. Coronal sections (300µm thick) were cut and placed onto the refrigerated stage. Three dopaminergic terminal fields were assayed: the mPFC, the CPu and the NAcc. For each structure, two or four tissue punches (1 mm diameter) from two consecutive sections were taken bilaterally and each side analyzed separately for the CPu, the NAcc and mPFC, respectively. Tissue punches were immersed into 50 µl of 0.1 N $HClO_4$ containing $Na_2S_2O_5$ (0,5%), disrupted by sonication and centrifuged at 15000 g for 20 min. Aliquots (10µl) of supernatant were diluted with high pressure liquid chromatography mobile phase and injected into a reverse-phase system consisting of a C18 column (HR-80 Catecholamine 80 x 4.6 mm, Thermo Scientific, USA) and a 0.1 M NaH_2PO_4 mobile phase containing 1-octanesulfonic acid (2.75 mM), triethylamine (0.25 mM), EDTA (0.1 mM), methanol (6 %) and adjusted to pH 2.9 with phosphoric acid. Flow rate was set at 0.6 mL/min by an ESA-580 pump. Electrochemical detection was performed with an ESA coulometric detector (Coulochem II 5100A, with a 5014B analytical cell; Eurosep, Cergy, France). The conditioning electrode was set at – 0.175 mV and the detecting electrode at + 0.175 mV, allowing a good signal-to-noise ratio. External standards were regularly injected to determine the elution times (9.8 and 21.5 min) and the sensitivity (0.3 and 0.4 pg), for DOPAC and DA respectively.

Data and materials availability. All data needed to evaluate the conclusions in the paper are present in the paper and/or Supplementary Materials. Additional data related to this paper may be requested from the authors

Author contributions

Conceived and designed the experiments: FT, SP, PF and DB. Performed the experiments: DB, CV, CN, SB, AZ, ACM, SM, AF, JPT and FM. Wrote the manuscript: FT, DB and SP and. Obtained funding and supervised the study: FT, PF and SP.

Funding sources

This project has been supported by grants from the LabEx BioPsy, the French Agence Nationale de la Recherche (ANR3053NEUR31438301 and ANR-14-CE35-0029-01), by the Foundation for Medical Research (FRM Equipe grant), and by the INCA.

Conflict of interest

We declare no conflict of interest.

Acknowledgements

We would like to thank F. Machulka and the IBPS mouse facility for their technical assistance.

Figure 1. Social hierarchy establishment and stability in mice.

(A) Design of social hierarchy establishment and analysis. Unfamiliar male mice were grouped by four. After 3 to 4 weeks, their social rank was determined by a precedence test (tube-test). **(B)** Rapidity of rank identification in the tube-test. The cumulated percentage of stable ranked individuals for each rank is pictured for each day of tube-test (n=60 tetrads). Gehan-Breslow-Wilcoxon Test (*p < 0.05; **p < 0.01; ***p < 0.001). **(C)** Mean duration of the confrontation in the tube-test performed during the three last days when rank was stable (n=48 tetrads). Each possible rank combination is pictured. For 14 tetrads, data from ranks 2 and 3 were omitted since they were still unstable. Wilcoxon rank sum test (*p < 0.05; **p < 0.01; ***p < 0.001). Error bars, +/- SEM. **(D)** Social ranks were stable over four months. The dynamic of social ranking in the tube-test is pictured for a set of 12 tetrads. Each line designs an individual mouse, its position within its social rank pool indicates the tetrad which it belongs to. Different colors indicate the rank defined at the first tube-test session. Individuals of ranks 2 and 3 that did not reach stability at the end of the first session are pictured with by black lines. A detailed figure, picturing daily results is available in supplementary data (Fig. S1). **(E)** Territorial urine marking reflects ranking obtained in tube-test. **Left**, representative picture of a urine marking during a 2 h confrontation between R1 and R4 mice, visualized with UV light. **Right**, contingency table of the ranking correspondences between the tube- and the urine marking-tests. Fisher's exact test, two-tailed, **p=0.003, n=23. **(F) Left**, representation of the warm spot test Position of the warm spot is pictured by an orange box. **Middle**, time course occupancy of the warm spot, total occupancy, and average length occupancy by differently ranked individuals (n=12 tetrads. **Right**, Representative occupancy periods of the warm spot by individuals of a tetrad.

Figure 2. Differences in social rank correlates with differences in behavior in genetically identical mice.

(A) Highest and lowest ranked animals display similar depression-like behavior in the forced-swim test. The length of immobility in tepid water is presented by 2 min periods (first row, mean central graph) and for the 6 min of the test (right) for R1 and R4 mice (n=11 per group). Results obtained on the first day (Day 1) and 24 h later (Day 2) are pictured. The lower row presents, in the same way, the quantification of escape behavior of the same individuals. Error bars, +/- SEM. **(B)** mice display increased anxiety-like behaviors. The experimental setups are pictured. The times spent for 48 R1 (green) and 48 R4 (red) individuals in the open-arm of an elevated O-maze and in the lit compartment of a dark-light box are pictured. Time was normalized from the R1 means. Respectively, t94=3.55, ***p<0.001 and t94=4.213, ****p<0.001, unpaired t-tests, two-tailed. Error bars, +/- SEM. **(C)** Highest ranked mice display increased sociability but

a social memory similar to that of lower ranked individuals. The three-chambers test is depicted. The times spent interacting with an empty box and with a box containing an unfamiliar mouse (Unfam.) are represented in the upper row for R1 (green boxes), R4 (red boxes) and both (black boxes). The times spent interacting with a box containing a familiar mouse (Fam.) *vs* an unfamiliar one (Unfam.) are represented in the lower row. Upper row: t114=6.012, **** p<0,0001, unpaired t-test, two-tailed. Right graph: effect of interaction, **** p<0.0001, $F_{(1,113)}$=17.07; effect of social cue, **** p<0.0001, $F_{(1,113)}$=41.7; no effect of social rank, p=0.97, $F_{(1,113)}$=0.002. Empty box: R1 *vs* empty box: R4, **p<0.01; Social cue R1 *vs* social cue R4, **p<0.01; Empty box *vs* social cue for R1 mice, **** p<0,0001. Empty box *vs* social cue for ranks 4 mice, p=0.20. Two-way mixed ANOVA, Bonferroni's test. Error bars, +/- SEM. Lower row : t114=15.19, **** p<0,0001, unpaired t-test, two-tailed. Right graph: no effect of interaction, p=0.30, $F_{(1,113)}$=1.097; effect of familiarity, **** p<0.0001, $F_{(1,113)}$=231.1; no effect of social rank, p=0.003, $F_{(1,113)}$=0.97. R1: familiar *vs* unfamiliar, ****p<0.0001; R4: familiar *vs* unfamiliar, ****p<0.0001. Two-way mixed ANOVA, Bonferroni's test. Error bars, +/- SEM. **(D)** Rank 1 individuals have better performances in a spatial working memory task. Upper row illustrates the task design. The learning curve of 11 mice from both ranks, indicates the progression of correct choices over the days (lower row, left). The number of days required to reach the learning criterion is pictured (mean and individual scores, right). Effect of time, ****p<0.0001, $F_{(3,60)}$=7.87; effect of social rank, * P=0.04, $F_{(1,20)}$=4.85; no effect of interaction, p=0.78, $F_{(3,60)}$=0.36. Two-way mixed ANOVA. Right panel indicates for each rank the average number of days required to acquire the criterion. U=34.5, p=0.056 Mann-Whitney u test, two tailed. Error bars, +/- SEM.

Figure 3. Differences in anxiety-like behavior does not pre-exist before colonization but differences in sociability does between future rank 1 and rank 4 individuals

(A) Future rank 1 and rank 4 individuals have similar despair behavior in a forced swim test. The time of immobility and escape time length are pictured for each periods of two minutes (lines), and for the six minutes of the test (bars). For future R1 (n=24), R4 (n=24), and R2/3 (n=48) (green, red, grey, respectively) Error bars, +/- SEM. **(B)** Future rank 1 and rank 4 individuals display similar anxiety-like behaviors. The time spent to explore the open segments of an elevated O-maze and the lit compartment of a dark-light box are pictured for all C57BL/6 mice (black bars, n=144 and n=192, respectively), and among them the future R1 (green bars, n=36 and n=48, respectively), the future R4 (red bars, n=36 and n=48, respectively) and the future ranks 2 and 3 (grey bars, n=72 and n=96, respectively). Scores are normalized from the R1 means. Error bars, +/- SEM. **(C**) Social behavior. Social preference (left), Future rank 4 mice did not show social preference unlike future R1 individuals. Duration of interactions with

an empty box *vs* a box containing an unfamiliar (Unfam.) congener is pictured for 136 mice C57BL/6 mice from tetrads (black bars) and, among them, the future R1 (green bars), R2/3 (grey bars) and R4 (red bars) (n=34, n=68, n=34, respectively). Left: t135=5.435, ****p<0.0001, unpaired t-test, two tailed. Right: no effect of interaction, p=0.09, $F_{(2,133)}$=2.41; effect of social cue, ****p<0.0001, $F_{(1,133)}$ 27.55; no effect of social rank, p=0.77, $F_{(2,133)}$=0.28. Empty box *vs* social cue for R1 mice, ****p<0.0001; empty box *vs* social cue for R2/3 mice, **p=0.001. Two-way mixed ANOVA, Bonferroni's test. Error bars, +/- SEM. Social memory. The time length interaction with the box containing a familiar (Fam.) social cue *vs* a box containing an unfamiliar (Unfam.) mouse is pictured. Left: t135=17.71, ****p<0.0001, unpaired t-test, two tailed. Right: no effect of interaction, p=0.01, $F_{(2,133)}$=2.31; effect of familiarity, ****p<0.0001, $F_{(1,133)}$=291.6; no effect of social rank, p=0.34, $F_{(2,133)}$=1.10. Familiar *vs* unfamiliar for R1 mice, ****p<0.0001; Familiar *vs* unfamiliar for R2/3 mice, ****p<0.0001; Familiar *vs* unfamiliar for R4 mice, ****p<0.0001. Unfamiliar social cue: R1 *vs* R4, *p=0.03. Two-way mixed ANOVA, Bonferroni's test. Error bars, +/- SEM.

Figure 4. Dopamine neurons activity in the ventral tegmental area varies with social rank.

(A) Left, schematic view of mesocorticolimbic system and electrode positioning (left). Representative traces of recording for individuals of each rank. Right, mean frequency (Hz) and percentage of spikes within bursts (SWB) of dopamine cells basal firing mice belonging to R1 (n=186, 10 individuals) and R4 (n=157, 10 individuals). For SWB data: t341=2.362, p*=0.02, unpaired t-test, two-tailed. Error bars, +/-SEM. **(B)** Sagittal representation sketches the section lines for tissue punches along the mesocorticolimbic pathway (bottom, coronal view). Dopamine release was quantified measuring the ration DOPAC/DA in the PFC, the NAcc, and the putamen caudate (CPu). For PFC, t22=3.256, **p<0.01, For PFC/NAcc t14=2.51, *p=0.025, unpaired t-tests, two-tailed. n represents the number of hemispheres for each group. Error bars, +/- SEM. VTA: ventral tegmental area, CPu: putamen caudate, PFC: prefrontal cortex, NAcc : nucleus accumbens.

Figure 5. R1 are less responsive to cocaine sensitization and more resilient to chronic stress

(A) Left panels, time course of locomotion for indicated sessions is pictured for R1 and R4 individuals (middle and right, respectively). Time 0 correspond to the injection of cocaine (Coc, 10mg kg^{-1}) or saline (Sal). Right panel, cumulated locomotor activity of R1 (n=8) and R1 (n=9) individuals (green and red lines, respectively) for 1 hour following habituation, saline (days 1

and 2) and cocaine (days 1 to 5 and a challenge on 12) injections. Effect of time, **** p<0.0001, $F_{(5,75)}$=7.55; effect of social rank, *p=0.035, $F_{(1,15)}$=5.376; no effect of interaction, p=0.32, $F_{(5,75)}$=1.198. Coc d12 R1 *vs* Coc d12 R4, *p=0.02. Two-way mixed ANOVA, Bonferroni's test. Error bars, +/- SEM. **(B)** Left panel, repeated social defeat protocol design. Middle left panel, representation the open-field in which social interactions were measured. The position of the box containing an unfamiliar CD1 mouse is indicated, as well are representative trajectories of R1 and R4 individuals before and after repeated social defeats. Middle right panel, R1 (green, n=8) and R4 (red, n=8) interaction time with an empty box (-) or a CD1 mouse (+), before and after repeated social defeat. Individual data are depicted. Right panel, susceptible individuals, developing social aversion are indicated with orange dots, resilient ones with blue dots. Pre-social defeats: effect of social cue, **** p<0.0001, $F_{(1,14)}$=41.2; no effect of social rank, p=0.39, $F_{(1,14)}$=0.76; no effect of interaction, p=0.33, $F_{(1,14)}$=1.04. Empty box *vs* social cue for R1 mice, *** p=0.0002; empty box *vs* social cue for R4 mice, **p=0.0038. Post-social defeats: no effect of social cue, p=0.19, $F_{(1,14)}$=1.89; no effect of social rank, p=0.60, $F_{(1,14)}$=0.29; effect of interaction, *p=0.04, $F_{(1,14)}$=4.95. Empty box *vs* social cue for R1 mice, *p=0.047. Two-way mixed ANOVA, Bonferroni's test. Error bars, +/- SEM. R1, n=8; R4, n=8.

Figure 6. GR deletion in dopaminoceptive neurons promotes higher social ranking in tetrads

Left, tetrads were constituted with one mutant (GR^{D1aCre}) and three control ($GR^{lox/lox}$) mice. The middle graph indicates the percentage of GR^{D1aCre} mice reaching each rank, among the 7 tetrads tested. The right graph pictures the total number of won test-tubes contests between GR^{D1aCre} and $GR^{lox/lox}$ mice, during the 3 last days of tube test. Fischer's exact test, two-tailed, ****p<0.0001, Error bars, +/- SEM.

Supplementary Figure 1. Stability of hierarchical classification over months

Social ranks were stable over several months. The dynamic of social ranking in the tube-test is pictured for a set of 12 tetrads. Each line corresponds to an individual mouse, its position within its social rank pool indicating the tetrad to which it belongs. For each individual, the color indicates its rank defined at the end of the first tube-test session (green, R1; blue, R2; yellow, R3; red, R4). Dots on the lines indicate that a tube-test was performed during the corresponding session. Note that there is no dot after that hierarchy has reached the stability criterion (*i.e.* that R1 and R4 mice were stable for three consecutive days of the series after an initial period of at least 6 days of testing. Black lines correspond to mice of intermediate ranks that did not reach stability at the end of the first session. In this example, tube-test series were repeated over 17 weeks.

Supplementary Figure 2. Basal locomotor activity in rank 1 and rank 4 individuals

Basal locomotor activity is pictured for R1 and R4 mice (n=12). Total distance traveled during 10 min, in an open-field box, is quantified in cm.

Supplementary Figure 3. Thymus and adrenal glands weighing

Weights in milligrams of both left and right adrenal glands, and thymus are represented for R1 and R4 mice (n=10).

References

Allsop, S.A., Vander Weele, C.M., Wichmann, R., Tye, K.M., 2014. Optogenetic insights on the relationship between anxiety-related behaviors and social deficits. Front Behav Neurosci 8.

Ambroggi, F., Turiault, M., Milet, A., Deroche-Gamonet, V., Parnaudeau, S., Balado, E., Barik, J., van der Veen, R., Maroteaux, G., Lemberger, T., Schütz, G., Lazar, M., Marinelli, M., Piazza, P.V., Tronche, F., 2009. Stress and addiction: glucocorticoid receptor in dopaminoceptive neurons facilitates cocaine seeking. Nature Neuroscience 12, 247–249.

Barik, J., Marti, F., Morel, C., Fernandez, S.P., Lanteri, C., Godeheu, G., Tassin, J.-P., Mombereau, C., Faure, P., Tronche, F., 2013. Chronic Stress Triggers Social Aversion via Glucocorticoid Receptor in Dopaminoceptive Neurons. Science 339, 332–335.

Barik, J., Parnaudeau, S., Saint Amaux, A.L., Guiard, B.P., Golib Dzib, J.F., Bocquet, O., Bailly, A., Benecke, A., Tronche, F., 2010. Glucocorticoid Receptors in Dopaminoceptive Neurons, Key for Cocaine, Are Dispensable for Molecular and Behavioral Morphine Responses. Biological Psychiatry 68, 231–239

Beery, A.K., Kaufer, D., 2015. Stress, social behavior, and resilience: Insights from rodents. Neurobiology of Stress 1, 116–127.

Bergmüller, R., Taborsky, M., 2010. Animal personality due to social niche specialisation. Trends in Ecology & Evolution 25, 504–511.

Berry, R.J., Bronson, F.H., 1992. Life History and bioeconomy of the House Mouse. Biological Reviews 67, 519–550.

Berton, O., McClung, C.A., DiLeone, R.J., Krishnan, V., Renthal, W., Russo, S.J., Graham, D., Tsankova, N.M., Bolanos, C.A., Rios, M., Monteggia, L.M., Self, D.W., Nestler, E.J., 2006. Essential Role of BDNF in the Mesolimbic Dopamine Pathway in Social Defeat Stress. Science 311, 864–868.

Bielsky, I.F., Hu, S.B., Szegda, K.L., Westphal, H., Young, L.J., 2004. Profound impairment in social recognition and reduction in anxiety-like behavior in vasopressin V1a receptor knockout mice. Neuropsychopharmacology. 29, 483-93.

Bjorklund A, Lindvall O., 1984. Dopamine containing systems in the CNS. In: Bjorklund A., Hokfelt T. (eds) Handbook of Chemical Neuroanatomy. Elsevier, Amsterdam, pp. 55–122

Cao, J.-L., Covington, H.E., Friedman, A.K., Wilkinson, M.B., Walsh, J.J., Cooper, D.C., Nestler, E.J., Han, M.-H., 2010. Mesolimbic Dopamine Neurons in the Brain Reward Circuit Mediate Susceptibility to Social Defeat and Antidepressant Action. J. Neurosci. 30, 16453–16458.

Cavigelli, S.A., Caruso, M.J., 2015. Sex, social status and physiological stress in primates: the importance of social and glucocorticoid dynamics. Philos Trans R Soc Lond B Biol Sci. 370, 1669

Cervenka, S., Gustavsson, J.P., Halldin, C., Farde, L., 2010. Association between striatal and extrastriatal dopamine D2-receptor binding and social desirability. NeuroImage 50, 323–328.

Chaudhury, D., Walsh, J.J., Friedman, A.K., Juarez, B., Ku, S.M., Koo, J.W., Ferguson, D., Tsai, H.-C., Pomeranz, L., Christoffel, D.J., Nectow, A.R., Ekstrand, M., Domingos, A., Mazei-Robison, M.S., Mouzon, E., Lobo, M.K., Neve, R.L., Friedman, J.M., Russo, S.J., Deisseroth, K., Nestler, E.J., Han, M.-H., 2013. Rapid regulation of depression-related behaviors by control of midbrain dopamine neurons. Nature 493, 532–536.

Cohen, S., Schwartz, J.E., Epel, E., Kirschbaum, C., Sidney, S., Seeman, T., 2006. Socioeconomic status, race, and diurnal cortisol decline in the Coronary Artery Risk Development in Young Adults (CARDIA) Study. Psychosom Med. 68, 41-50.

Cordero, M.I., Sandi, C., 2007. Stress amplifies memory for social hierarchy. Front Neurosci. 1, 175-84.

Creel, S., Dantzer, B., Goymann, W., Rubenstein, D.R., 2012. The ecology of stress: effects of the social environment. Funct. Ecol. 27, 66–80.

Felix-Ortiz, A. C., Beyeler, A., Seo, C., Leppla, C.A., Wildes, C.P., Tye, K.M., 2013. BLA to vHPC inputs modulate anxiety-related behaviors. Neuron 79, 658–664.

Felix-Ortiz, A.C., Tye, K.M., 2014. Amygdala inputs to the ventral hippocampus bidirectionally modulate social behavior. J. Neurosci. 34, 586–595.

Ferrari, P.F., Palanza, P., Parmigiani, S., Rodgers, R.J., 1998. Interindividual variability in Swiss male mice: relationship between social factors, aggression, and anxiety. Physiol. Behav. 63, 821–827.

Francis, R.C., 1984. The Effects of Bidirectional Selection for Social Dominance On Agonistic Behavior and Sex Ratios in the Paradise Fish (Macropodus Opercularis). Behavior 90, 25–44.

Freund, J., Brandmaier, A.M., Lewejohann, L., Kirste, I., Kritzler, M., Krüger, A., Sachser, N., Lindenberger, U., Kempermann, G., 2013. Emergence of Individuality in Genetically Identical Mice. Science 340, 756–759.

Grace, A.A., Bunney, B.S., 1984. The control of firing pattern in nigral dopamine neurons: burst firing. J. Neurosci. 4, 2877–2890.

Gunaydin, L.A., Grosenick, L., Finkelstein, J.C., Kauvar, I.V., Fenno, L.E., Adhikari, A., Lammel, S., Mirzabekov, J.J., Airan, R.D., Zalocusky, K.A., Tye, K.M., Anikeeva, P., Malenka, R.C., Deisseroth, K., 2014. Natural neural projection dynamics underlying social behavior. Cell 157, 1535–1551.

Hager, T., Jansen, R.F., Pieneman, A.W., Manivannan, S.N., Golani, I., van der Sluis, S., Smit, A.B., Verhage, M., Stiedl, O., 2014. Display of individuality in avoidance behavior and risk assessment of inbred mice. Front Behav Neurosci 8:314.

Hilakivi, L.A., Lister, R.G., Durcan, M.J., Ota, M., Eskay, R.L., Mefford, I., Linnoila, M., 1989. Behavioral, hormonal and neurochemical characteristics of aggressive alpha-mice. Brain Res. 502, 158–166.

Hilakivi-Clarke, L.A., Lister, R.G., 1992. Are there preexisting behavioral characteristics that predict the dominant status of male NIH Swiss mice (Mus musculus)? Journal of Comparative Psychology 106, 184–189.

Hollis, F., van der Kooij, M.A., Zanoletti, O., Lozano, L., Cantó, C., Sandi, C., 2015. Mitochondrial function in the brain links anxiety with social subordination. Proceedings of the National Academy of Sciences 112, 15486–15491.

Insel, T.R., 2010. The Challenge of Translation in Social Neuroscience: A Review of Oxytocin, Vasopressin, and Affiliative Behavior. Neuron 65, 768–779.

Jupp, B., Murray, J.E., Jordan, E.R., Xia, J., Fluharty, M., Shrestha, S., Robbins, T.W., Dalley, J.W., 2016. Social dominance in rats: effects on cocaine self-administration, novelty reactivity and dopamine receptor binding and content in the striatum. Psychopharmacology (Berl) 233, 579–589.

Kessler, R.C., McGonagle, K.A., Zhao, S., Nelson, C.B., Hughes, M., Eshleman, S., Wittchen, H.U., Kendler, K.S., 1994. Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Results from the National Comorbidity Survey. Arch. Gen. Psychiatry 51, 8–19.

Korzan, W.J., Forster, G.L., Watt, M.J., Summers, C.H., 2006. Dopaminergic activity modulation via aggression, status, and a visual social signal. Behav. Neurosci. 120, 93–102.

Kunkel, T., Wang, H., 2018. Socially dominant mice in C57BL6 background show increased social motivation. Behav. Brain Res. 336, 173–176.

Larrieu, T., Cherix, A., Duque, A., Rodrigues, J., Lei, H., Gruetter, R., Sandi, C., 2017. Hierarchical Status Predicts Behavioral Vulnerability and Nucleus Accumbens Metabolic Profile Following Chronic Social Defeat Stress. Current Biology 27, 2202-2210.e4.

Lathe, R., 2004. The individuality of mice. Genes, Brain and Behavior 3, 317–327.

Lehmann, M.L., Geddes, C.E., Lee, J.L., Herkenham, M., 2013. Urine Scent Marking (USM): A Novel Test for Depressive-Like Behavior and a Predictor of Stress Resiliency in Mice. PLoS One 8(7):e69822

Lemberger, T., Parlato, R., Dassesse, D., Westphal, M., Casanova, E., Turiault, M., Tronche, F., Schiffmann, S.N., Schütz, G., 2007. Expression of Cre recombinase in dopaminoceptive neurons. BMC Neurosci 8, 4.

Lorant, V., 2003. Socioeconomic Inequalities in Depression: A Meta-Analysis. American Journal of Epidemiology 157, 98–112. https://doi.org/10.1093/aje/kwf182

Lozano-Montes, L., Astori, S., Abad, S., Guillot de Suduiraut, I., Sandi, C., Zalachoras, I., 2019. Latency to Reward Predicts Social Dominance in Rats: A Causal Role for the Dopaminergic Mesolimbic System. Front Behav Neurosci 13.69

Martinez, D., Orlowska, D., Narendran, R., Slifstein, M., Liu, F., Kumar, D., Broft, A., Van Heertum, R., Kleber, H.D., 2010. Dopamine type 2/3 receptor availability in the striatum and social status in human volunteers. Biol. Psychiatry 67, 275–278.

McIntyre, D.C., Chew, G.L., 1983. Relation between social rank, submissive behavior, and brain catecholamine levels in ring-necked pheasants (Phasianus colchicus). Behav. Neurosci. 97, 595–601.

Miller-Butterworth, C.M., Kaplan, J.R., Shaffer, J., Devlin, B., Manuck, S.B., Ferrell, R.E., 2008. Sequence Variation in the Primate Dopamine Transporter Gene and Its Relationship to Social Dominance. Mol Biol Evol 25, 18–28.

Nader, M.A., Nader, S.H., Czoty, P.W., Riddick, N.V., Gage, H.D., Gould, R.W., Blaylock, B.L., Kaplan, J.R., Garg, P.K., Davies, H.M.L., Morton, D., Garg, S., Reboussin, B.A., 2012. Social dominance in female monkeys: dopamine receptor function and cocaine reinforcement. Biol. Psychiatry 72, 414–421.

Okada, Y., Sasaki, K., Miyazaki, S., Shimoji, H., Tsuji, K., Miura, T., 2015. Social dominance and reproductive differentiation mediated by dopaminergic signaling in a queenless ant. Journal of Experimental Biology 218, 1091–1098.

Papilloud, A., Weger, M., Bacq, A., Zalachoras, I., Hollis, F., Larrieu, T., Battivelli, D., Grosse, J., Zanoletti, O., Parnaudeau, S., Tronche, F., Sandi, C., 2019. The glucocorticoid receptor in the nucleus accumbens plays a crucial role in social rank attainment in rodents. bioRxiv. doi: 10.1101/668897

Penick, C.A., Brent, C.S., Dolezal, K., Liebig, J., 2014. Neurohormonal changes associated with ritualized combat and the formation of a reproductive hierarchy in the ant Harpegnathos saltator. Journal of Experimental Biology 217, 1496–1503.

Robinson, T.E., Berridge, K.C., 2000. The psychology and neurobiology of addiction: an incentive–sensitization view. Addiction 95, 91–117.

Robison, A.J., Nestler, E.J., 2011. Transcriptional and epigenetic mechanisms of addiction. Nature Reviews Neuroscience 12, 623–637.

Rodriguiz, R.M., Chu, R., Caron, M.G., Wetsel, W.C., 2004. Aberrant responses in social interaction of dopamine transporter knockout mice. Behavioral Brain Research 148, 185–198.

Runegaard, A.H., Sørensen, A.T., Fitzpatrick, C.M., Jørgensen, S.H., Petersen, A.V., Hansen, N.W., Weikop, P., Andreasen, J.T., Mikkelsen, J.D., Perrier, J.-F., Woldbye, D., Rickhag, M., Wortwein, G., Gether, U., 2018. Locomotor- and Reward-Enhancing Effects of Cocaine Are Differentially Regulated by Chemogenetic Stimulation of Gi-Signaling in Dopaminergic Neurons. eNeuro 5.(3)

Russo, S.J., Murrough, J.W., Han, M.-H., Charney, D.S., Nestler, E.J., 2012. Neurobiology of resilience. Nature Neuroscience 15, 1475–1484.

Sapolsky, R.M., 2004. Social status and health in humans and other animals. Ann Rev. Anthropol 33, 393-418

Sapolsky, R.M., 2005. The influence of social hierarchy on primate health. Science 308, 648–652.

Sherman, G.D., Lee, J., Cuddy, A., Renshon, J., Oveis, C., Gross, J., Lerner, J. 2012. Leadership is associated with lower levels of stress. Proceedings of the National Academy of Sciences 109, 17903-17907

Singh-Manoux, A., Marmot, M., Adler, N., 2005. Does Subjective Social Status Predict Health and Change in Health Status Better Than Objective Status? Psychosomatic Medicine 67, 855–861.

Sigurdsson, T., Stark, K.L., Karayiorgou, M., Gogos, J.A., Gordon, J.A., 2010. Impaired hippocampal–prefrontal synchrony in a genetic mouse model of schizophrenia. Nature 464, 763–767.

Starr-Phillips, E.J., Beery, A.K., 2014. Natural variation in maternal care shapes adult social behavior in rats. Developmental Psychobiology 56, 1017–1026.

Timmer, M., Sandi, C., 2010. A role for glucocorticoids in the long-term establishment of a social hierarchy. Psychoneuroendocrinology 35, 1543–1552.

Tinbergen, N., 1939. On the Analysis of Social Organization Among Vertebrates, with Special Reference to Birds. The American Midland Naturalist 21, 210–234.

Torquet, N., Marti, F., Campart, C., Tolu, S., Nguyen, C., Oberto, V., Benallaoua, M., Naudé, J., Didienne, S., Debray, N., Jezequel, S., Le Gouestre, L., Hannesse, B., Mariani, J., Mourot, A., Faure, P., 2018. Social interactions impact on the dopaminergic system and drive individuality. Nature Communications 9(1):3081.

Tye, K.M., Prakash, R., Kim, S.-Y., Fenno, L.E., Grosenick, L., Zarabi, H., Thompson, K.R., Gradinaru, V., Ramakrishnan, C., Deisseroth, K., 2011. Amygdala circuitry mediating reversible and bidirectional control of anxiety. Nature 471, 358–362.

van der Kooij, M.A., Hollis, F., Lozano, L., Zalachoras, I., Abad, S., Zanoletti, O., Grosse, J., Guillot de Suduiraut, I., Canto, C., Sandi, C., 2018. Diazepam actions in the VTA enhance social dominance and mitochondrial function in the nucleus accumbens by activation of dopamine D1 receptors. Mol Psychiatry 23, 569–578.

Varholick, J.A., Bailoo, J.D., Palme, R., Würbel, H., 2018. Phenotypic variability between Social Dominance Ranks in laboratory mice. Sci Rep 8.

von Rueden, C.R., Trumble, B.C., Emery Thompson, M., Stieglitz, J., Hooper, P.L., Blackwell, A.D., Kaplan, H.S., Gurven, M., 2014. Political influence associates with cortisol and health among egalitarian forager-farmers. Evol Med Public Health. 1, 122-133.

Wang, F., Zhu, J., Zhu, H., Zhang, Q., Lin, Z., Hu, H., 2011. Bidirectional Control of Social Hierarchy by Synaptic Efficacy in Medial Prefrontal Cortex. Science 334, 693–697.

Watson, C., Paxinos, G. (2010). Chemoarchitectonic Atlas of the Mouse Brain. San Diego, Elsevier Academic Press

Wilkinson, R.G., 1999. Health, hierarchy, and social anxiety. Ann. N. Y. Acad. Sci. 896, 48–63.

Yamaguchi, Y., Lee, Y.-A., Goto, Y., 2015. Dopamine in socioecological and evolutionary perspectives: implications for psychiatric disorders. Frontiers in Neuroscience 9:219.

Yamaguchi, Y., Lee, Y.-A., Kato, A., Jas, E., Goto, Y., 2017. The Roles of Dopamine D2 Receptor in the Social Hierarchy of Rodents and Primates. Scientific Reports 7:43348.

Yamaguchi, Y., Lee, Y.-A., Kato, A., Goto, Y., 2017. The Roles of Dopamine D1 Receptor on the Social Hierarchy of Rodents and Non-Human Primates. International Journal of Neuropsychopharmacology 20(4):324-335.

Zhou, T., Sandi, C., Hu, H., 2018. Advances in understanding neural mechanisms of social dominance. Current Opinion in Neurobiology 49, 99–107.

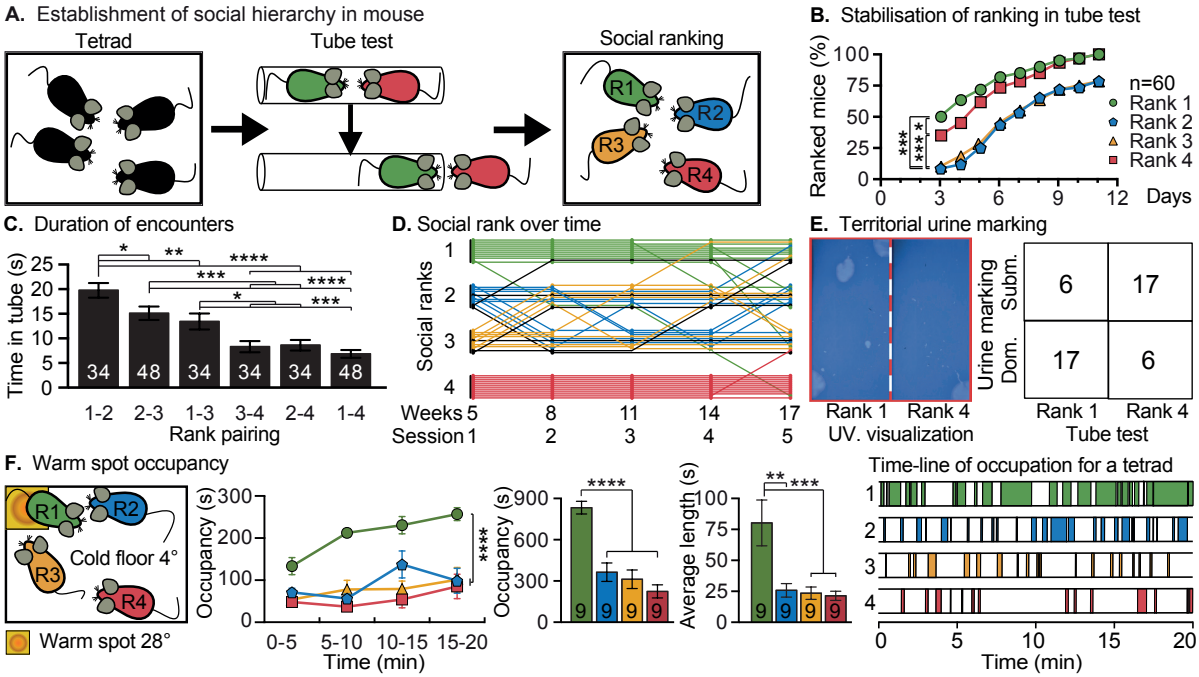


Figure 1

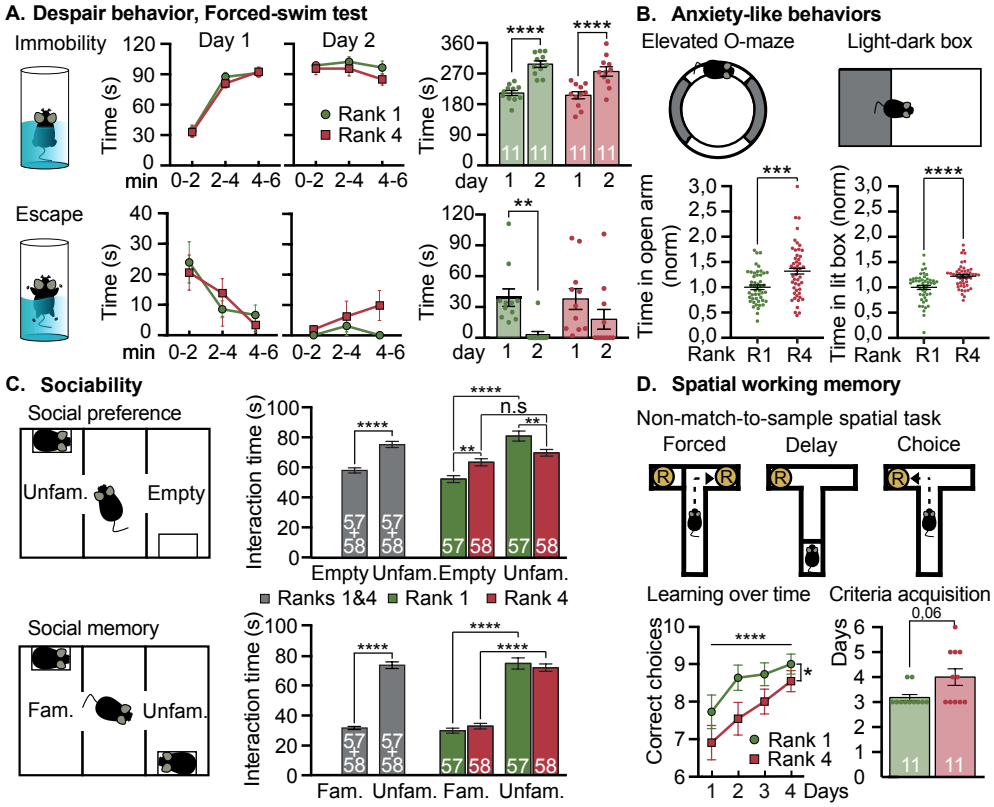


Figure 2

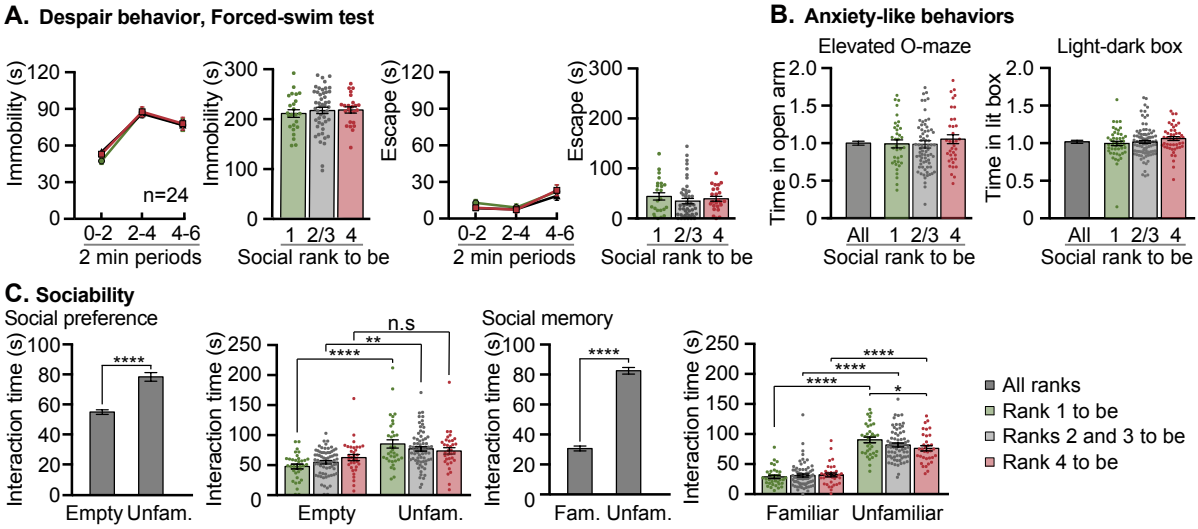


Figure 3

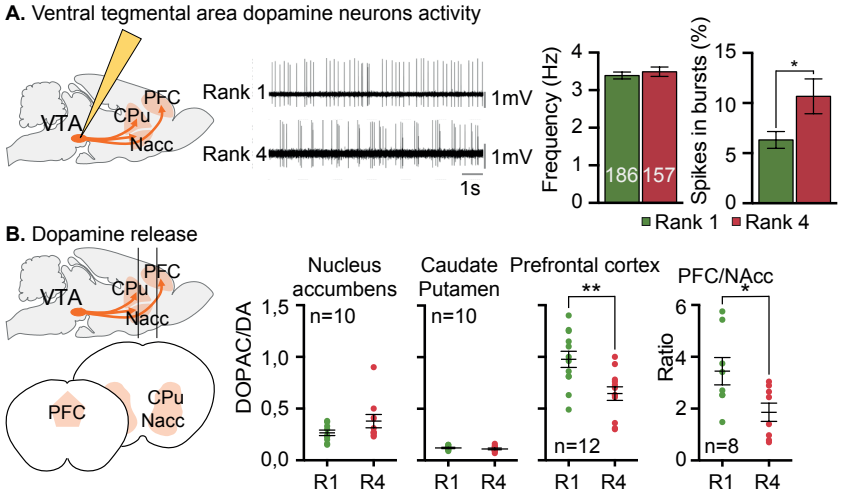


Figure 4

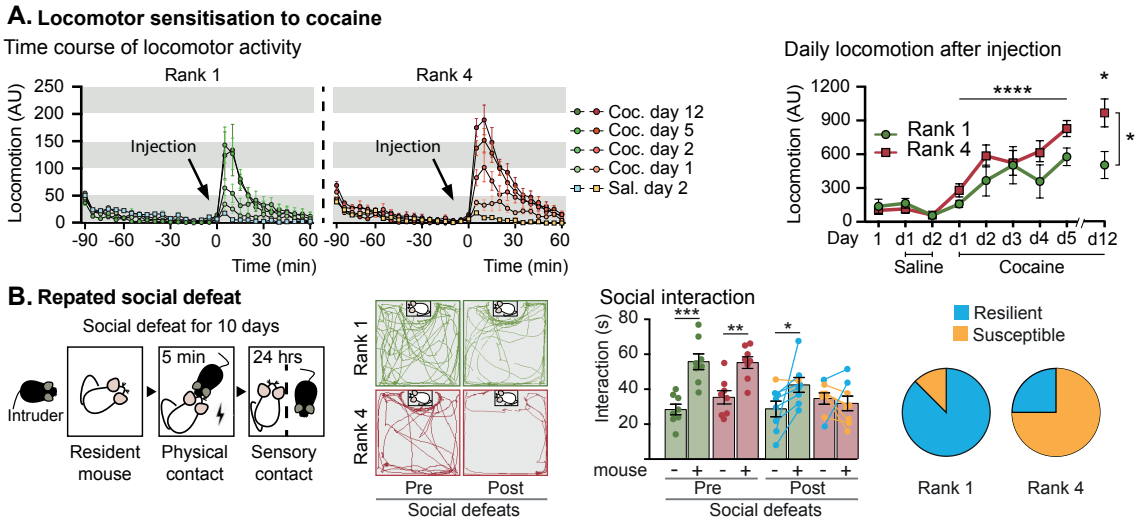


Figure 5

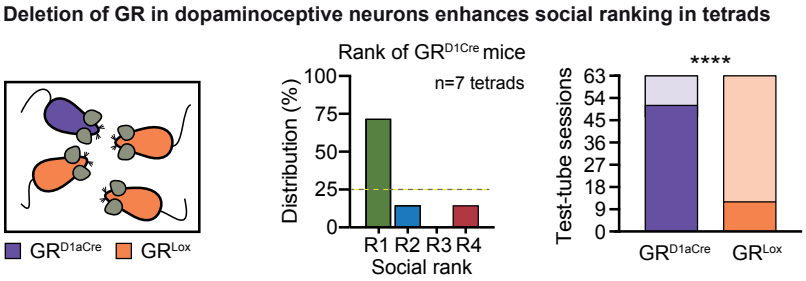
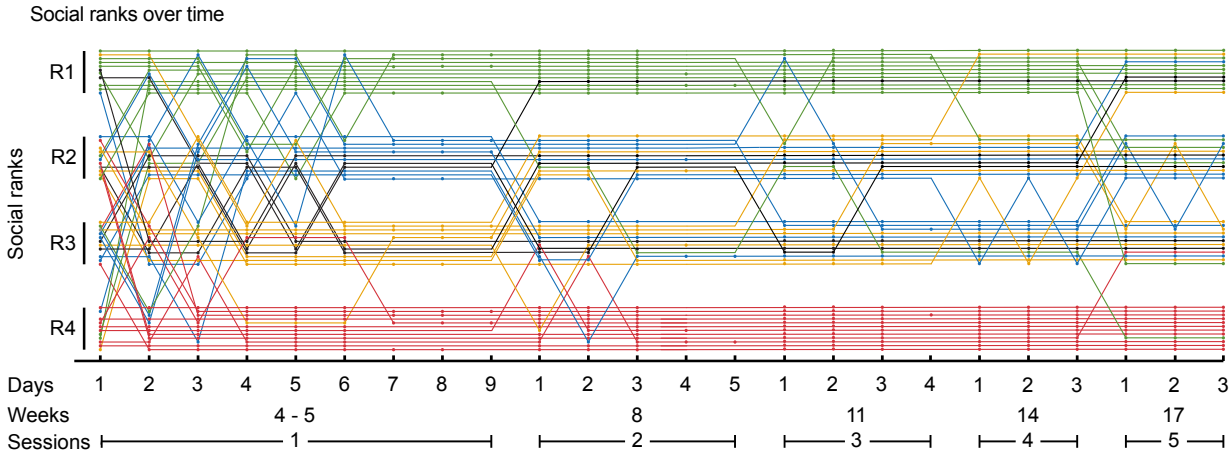
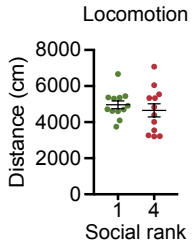


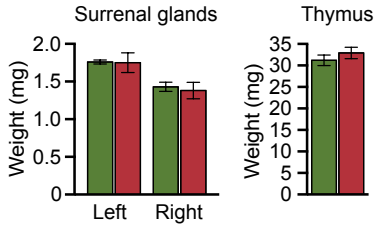
Figure 6



Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3