



**HAL**  
open science

## **Dopamine neuron activity and stress signalling as links between social hierarchy and psychopathology vulnerability**

Dorian Battivelli, Cécile Vernochet, Estelle Conabady, Claire Nguyen, Abdallah Zayed, Ashley Lebel, Aura Carole Meirsman, Sarah Messaoudene, Alexandre Fieggen, Gautier Dreux, et al.

### ► To cite this version:

Dorian Battivelli, Cécile Vernochet, Estelle Conabady, Claire Nguyen, Abdallah Zayed, et al.. Dopamine neuron activity and stress signalling as links between social hierarchy and psychopathology vulnerability. *Biological Psychiatry*, 2024, 95 (8), pp.774-784. 10.1016/j.biopsych.2023.08.029 . hal-04284656v2

**HAL Id: hal-04284656**

**<https://hal.science/hal-04284656v2>**

Submitted on 28 Nov 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

**Dopamine neuron activity and stress signaling as links between social hierarchy and psychopathology vulnerability.**

Dorian Battivelli <sup>1,2,3</sup>, Cécile Vernochet <sup>1,2,3</sup>, Estelle Conabady<sup>1,2,3</sup>, Claire Nguyen <sup>2,3,4</sup>, Abdallah Zayed <sup>1,2,3</sup>, Ashley Lebel <sup>1,2,3</sup>, Aura Carole Meirsman <sup>1,2,3</sup>, Sarah Messaoudene <sup>1,2,3</sup>, Alexandre Fieggen <sup>1,2,3</sup>, Gautier Dreux <sup>1,2,3</sup>, Daiana Rigoni<sup>5,6</sup>, Tinaïg Le Borgne <sup>2,3,4</sup>, Fabio Marti <sup>2,3,4</sup>, Thomas Contesse <sup>5,6</sup>, Jacques Barik <sup>5,6</sup>, Jean-Pol Tassin <sup>1,2,3</sup>, Philippe Faure <sup>2,3,4</sup>, Sébastien Parnaudeau <sup>1,2,3,\*</sup>, †, François Tronche <sup>1,2,3,\*</sup>, †

<sup>1</sup> Gene Regulation and Adaptive Behaviours group, Neuroscience Paris Seine, UMR8246, Centre National de la Recherche Scientifique (CNRS), Institut de Biologie Paris Seine, 75005, Paris, France.

<sup>2</sup> INSERM U1130.

<sup>3</sup> Sorbonne Université UMCR18,

<sup>4</sup> Neurophysiology and Behaviour group, Neuroscience Paris Seine, UMR8246, CNRS.

<sup>5</sup> Université Côte d'Azur, Nice, France

<sup>6</sup> Institut de Pharmacologie Moléculaire & Cellulaire, CNRS UMR7275, Valbonne, France.

\* Corresponding authors: François Tronche ([francois.tronche@sorbonne-universite.fr](mailto:francois.tronche@sorbonne-universite.fr)) and Sébastien Parnaudeau ([sebastien.parnaudeau@sorbonne-universite.fr](mailto:sebastien.parnaudeau@sorbonne-universite.fr))

† These authors contributed equally to this work.

## **Abstract**

### **Background**

Social status in human, generally reflected by socioeconomic status, has been associated, when constrained, with heightened vulnerability to pathologies including psychiatric diseases. Social hierarchy in mice translates into individual and interdependent behavioral strategies of animals within a group. The rules leading to the emergence of a social organization are elusive and detangling the contribution of social status from other factors, whether environmental or genetic, to normal and pathological behaviors remains challenging.

### **Methods**

We investigated the mechanisms shaping the emergence of a social hierarchy in isogenic C57Bl/6 mice raised by groups of four using conditional mutant mouse models and chemogenetic manipulation of dopamine midbrain neuronal activity. We further studied the evolution of behavioral traits and the vulnerability to psychopathological-like phenotypes according to the social status of the animals.

### **Results**

Higher sociability predetermined higher social hierarchy in the colony. Upon hierarchy establishment, higher ranked mice showed increased anxiety and better cognitive abilities in a working memory task. Strikingly, the higher ranked mice displayed a reduced activity of dopaminergic neurons within the ventral tegmental area, paired with a decrease behavioral response to cocaine and a decreased vulnerability to depressive-like behaviors following repeated social defeats. The pharmacogenetic inhibition of this neuronal population and the genetic inactivation of glucocorticoid receptor signaling in dopamine-sensing brain areas that resulted in decreased dopaminergic activity promoted the accession to higher social ranks.

### **Conclusions**

Dopamine activity and its modulation by the stress response shapes social organization in mice, potentially linking interindividual and social status differences in vulnerability to 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 (1, 2). 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 emergence of individuality that distinguishes it from others (3, 4) and translates into repeated and consistent strategies to find food, deal with predators, or compete with conspecifics.

Mice are social vertebrates, living in hierarchical structures of 4 to 12 members (5, 6) that share territorial defense and exhibit a large repertoire of social behaviors, including physical exploration, vocal communication, aggression, imitation, *etc.* The social rank of individuals can be determined based on precedence behaviors, access to limited resources or antagonistic interactions (7). The driving forces patterning the emergence of social organization remain largely unknown. Although these include genetic factors, the fact that isogenic congeners form a hierarchy suggests that environmental factors are in play. Stress exposure and glucocorticoids release, for example seem to influence social dominance in a variety of species, although a clear link has yet to be drawn (8, 9).

Hierarchy establishment involves iterative pairwise interactions and have consequences for the individual behavioral fate (10, 11). In congenic mice, specific behavioral patterns emerge in mice raised in large groups (12, 13), and hierarchy-based differences in behavioral traits are observed in smaller colonies (14, 15). Whether such individual differences pre-exist 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 of animals, these questions are also relevant in humans, in a psychopathological context, since low social status is recognized as a vulnerability risk factor for psychopathologies, including mood disorders and addiction (16, 17).

The mesocorticolimbic system that encompasses the prefrontal cortex (PFC), the nucleus accumbens (NAcc) and their dopaminergic input from the ventral tegmental area (VTA) may participate in the emergence of social hierarchy and behavioral diversity and may constitute a link between social status and mental health. This brain system modulates a broad spectrum of behaviors, including motivation and decision-making involved in social context (18). The activity of VTA dopamine neurons conditions

social avoidance following social defeats, a preclinical model of depression (19, 20). The interaction between stress-evoked release of glucocorticoids and the dopamine system is critical for this effect and relies on the activation of glucocorticoid receptors (GR) present in neurons of dopamine-sensing brain areas (20). Several structures receiving dopaminergic inputs have been associated with social ranking. Modulating the synaptic efficacy in medial PFC neurons causes individual bidirectional shifts in ranking (14), and inputs from the thalamus to the medial PFC along with output from the PFC to hypothalamic regions seem key in the establishment of social rank (21, 22). Finally, lower mitochondrial activity within the NAcc is associated to lower social ranking in both rats and mice (15, 23). Despite the evidence pointing to a role for brain regions belonging to the meso-corticolimbic reward system in the social hierarchy, the contribution of VTA dopamine neurons themselves has never been investigated.

In this study, we examine the segregation of individual behaviors with social status in colonies of four genetically identical male mice (tetrads). We investigated whether pre-existing behavioral and physiological differences shape the social fate of individuals, or whether such differences emerge from social life. Finally, we provide evidence for an implication of the mesocorticolimbic dopamine system and stress response signaling in the establishment of social hierarchy and individuation of behaviors.

## **Methods and Materials**

### **Animals**

Experiments were performed on C57BL/6JRj mice purchased at 6 weeks old. GR<sup>D1Cre</sup> and BAC-DATiCre<sup>flo</sup> mice were used for the analysis of GR function and of dopamine neurons activity respectively.

### **Social ranking and behaviors**

Mice were housed in tetrads for 2 to 4 weeks before rank testing, done using the precedence tube test.

### **Recordings and manipulation of dopamine neurons**

Dopamine neurons activity was assessed using extracellular recordings in anesthetized mice. and was decreased using a pharmacogenetic approach (hM4D).

Methods are detailed in supplementary information.

## **Results**

### **Social ranks within tetrads are stable over long periods**

We analyzed social ranks and behaviors in tetrad colonies of four weights matched six to eight weeks-old C57B/L6 individuals previously unknown to each other (see Figure S1 for the timeline of the experiments). Two to four weeks following tetrad formation, we analyzed the social ranks of animals using a precedence test based on encounters within a plastic tube between each possible congener pair among a tetrad. Higher-ranked individuals come out of the tube walking forward (14) (Figure 1A). We tested nine times a day the six possible pairwise combinations of individuals from a tetrad. The individual with the highest number of forward exits was classified as higher ranked. We tested each tetrad, until the measurements of the highest (rank 1, R1) and lowest ranks (rank 4, R4) were stable over 3 consecutive days.

Among 60 tetrads, the stability criterion was reached faster for the extreme ranks (Figure 1B), and as observed by Wang *et al.* (14), the rank of individuals conditioned the duration of contests. Confrontations between R1 and R2 individuals lasted for an average of 19.4 seconds whereas confrontations involving R4 lasted twice less (Figure 1C).

Once established, social ranking was stable over long periods. Figure 1D represents the social fate of individuals from 12 tetrads repeatedly assessed over four months. This is particularly true for R4

individuals as 17 weeks later, eleven out of twelve mice remained at the lowest rank. Among initially highest ranked individuals, seven kept the same ranking (Figure 1D and S2A). The highest number of switches was observed in animals initially intermediately ranked (R2 and R3), however 19 out of 24 were still at an intermediate rank 17 weeks later (Figure 1D). Of note, no correlation between social rank and weight evolution was found (data not shown).

To validate precedence behavior as a reliable proxy for social ranking, we quantified other expressions of social dominance. We first assessed territoriality by analyzing urine marking patterns in R1 and R4 individuals placed in an open-field, separated by a plastic wall. We did not find significant differences for the number of marks, their cumulated area, or their distance from the separation. We then examined the access to shared resources measuring the occupancy of a small warm spot within a cold cage (21) (Figure 1E). We found that R1 mice identified in the tube-test spent significantly more time on the warm spot during the 20 minutes of the test compared to their cage-mates, with 3 to 4 times longer episodes of occupancy thus confirming their higher rank in the hierarchy.

Finally, literature often relies on aggressiveness to determine social dominance. To assess potential differences in aggressive behaviors between the R1 and R4 individuals, we performed a resident-intruder test, repeated for two consecutive days with R1 and R4 single-housed mice for 48h. For half of them, at least one R1 or R4 mouse attacked the intruder. For these pairs, we quantified the interactions with the intruder (Figure S3). On the first day, R4 individuals displayed significantly more aggressive behaviors, including clinch attacks, lateral threats, chases, *etc.* whereas R1 individuals had more prosocial behaviors (sniffing, grooming). On the second day, this difference was even more pronounced, none of the R1 individuals attacked the intruder whereas all R4 mice did so within the first 130 seconds.

### **Social rank correlates with behavioral differences.**

Behaviors tested in laboratory settings on isogenic mice, including anxiety-like, sociability, or locomotion display high interindividual variability, probably resulting from a combination of environmental factors. To address the potential contribution of social ranking to this, we compared R1 and R4 individuals. We did not notice any differences in locomotor activity, measured in an open-field (data not shown), or in stress-coping, measured by quantifying immobility and escape in the forced-swim test (Figure S4A). When looking at anxiety, stratification between R1 and R4 mice reveals a higher anxiety profile in highest-ranked individuals in both elevated O-maze and dark-light tests (Figure 2A). We also compared

sociability and social memory between R1 and R4 mice in a three-chamber test. As expected, C57BL/6 mice display a marked preference for a social stimulus (Figure 2B, grey bars). Stratification of the results shows that only R1 individuals, and not R4, displayed a social stimulus preference (Figure 2B, blue bars). Social rank does not affect social memory or preference for social novelty. Both ranks had a similar preference for interacting with an unfamiliar conspecific vs. a familiar one (Figure S4B). Overall, these data show that R1 mice are more anxious and more sociable than R4 ones. To address whether the individuals with highest anxiety also the more sociable, we made a correlation analysis in either R1, R4 mice, or in all mice pooled together, but did not find any (Figure S5A), suggesting that the differences observed in these two behaviors could be driven by different populations of mice.

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

The behavioral differences observed between ranks could emerge from social life. Alternatively, they could pre-exist and shape individual social ranking trajectories. To address this question, we compared individual behaviors before, and after the formation of the social colony (Figure 2C). The higher anxiety-like of R1 individuals seems to emerge from the social organization since no difference was observed between future R1 and R4 mice (Figure 2D). Looking at the evolution of anxiety before and during life in colony, we found that while R4 mice tended to see anxiety level decreasing with social rank establishment, the opposite was true for R1 mice (Figure S5B).

In contrast, differences in sociability pre-exist to life in colony. Future R1 mice already showed a marked interest in social interactions before social life, as observed once the tetrad was formed, whereas future R4 did not (Figure 2E and S5C). Intermediate ranks have an intermediate phenotype with a significant but lower preference. Concerning social memory, although we did not observe difference between established R1 and R4 individuals, future R1 mice might display a slightly better performance than future R4 ones (Figure S6A). As expected, we did not observe any difference in despair-like behavior between future R1 and R4 individuals (Figure S6B).

### **Social rank conditions sensitivity to psychopathology-like behaviors**

In human, low social status, is associated with reduced life span (24) and negative health consequences including psychopathologies. In several social vertebrate species, associations between social rank and



health outcomes have been documented (25). We investigated whether highest- and lowest-ranked individuals would respond distinctly to preclinical models of mental disorders in mice.

The locomotor sensitization to cocaine is a gradual and enduring facilitation of locomotor activity with repeated cocaine exposure, reflecting the reinforcing effects of abused drugs (26). Locomotor response to daily cocaine injections ( $10 \text{ mg.kg}^{-1}$ ) was higher in R4 than in R1 mice (Figure 3A). This remained stable after a withdrawal period of 7 days, R4 mice appeared more sensitive to a challenge injection (Figure 3A, day 12). R1 mice reached the same levels of locomotor response than R4 mice only when giving them a higher dose of cocaine on the day of challenge 2 confirming their lower sensitivity to cocaine (Figure 3A, day 13).

Repeated social defeats is a well-validated mouse model of depression, marked by an enduring social aversion, which allows to distinguish animals that exhibit depressive-like symptoms (susceptible) from those which are resilient to stress (27). The highest- and lowest-ranked mice from eight tetrads were daily subjected to social defeats for 10 consecutive days by an unfamiliar aggressor and remained in sensory (but not physical) contact for the rest of the day (Figure 3B, left panel). We quantified in an open-field the time of interaction with an empty plastic box vs. a box containing an unfamiliar male mouse, without and after social defeat (Figure 3B middle). 7 out of the 16 mice challenged developed a social aversion (Figure 3B, right, orange lines). Among them, only one was an R1 individual. 87.5% of R1 individuals were resilient but only 25% of R4 ones (Figure 3B, right).

Working memory is a key cognitive function commonly compromised in psychopathologies (28). We investigated in a non-match-to-sample T-maze task whether social ranking could affect it (Figure 3C, left). In this task, mice are placed within a T-Maze and can access a reward positioned 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 to find 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. Both groups of mice learned the task, but R1 individuals did it significantly faster than R4 indicating better working memory abilities (Figure 3C, right).

**Reduced activity of VTA dopamine neurons facilitates higher ranking.**

We previously showed that GR gene inactivation in dopamine-innervated areas facilitates resilience to social defeat by preventing and reduces responses to cocaine (20, 29, 30). Interestingly, these phenotypes are associated with lower VTA dopamine neuron activity (29, 20). We thus investigated whether differences in VTA dopamine neuron activity could exist between R1 and R4 individuals. We performed juxtacellular single-unit recordings in anesthetized mice (Figure 4A, left). The analysis of 186 neurons from 10 R1 mice and 157 neurons from 10 R4 mice revealed that, whereas the frequency of spontaneous firing was similar in both ranks (Figure 4A, left graph), the percentage of spikes within bursts was significantly lower in R1 individuals (Figure 4A, right graph).

We then studied whether GR signaling within the dopamine reward pathway could influence social ranking in tetrads. We grouped one adult GR<sup>D1aCre</sup> mice with three unfamiliar control (GR<sup>loxP/loxP</sup>) individuals (Figure 4B, left panel) and assessed their social rank in the tube-test two weeks later. Over the last three days of rank stabilization, GR<sup>D1aCre</sup> mice displayed a significantly higher average of daily wins compared to controls (Figure 4B, left chart). During this period, GR<sup>D1aCre</sup> mice accumulated a total number of 139 wins out of 189 contests against control mice, which corresponds to a probability of about 4.9% for a randomly selected mouse in a tetrad to have more or the same number of wins (Figure 4B, middle chart). This increase in the number of won contests is reflected in the attained social rank. In 5 out of 7 tetrads, GR<sup>D1aCre</sup> mice ended up in the highest rank, meaning that GR<sup>D1aCre</sup> mice have a significantly higher chance to reach the highest social rank (Figure 4B, right chart).

### **Reduced activity of VTA dopamine neurons increases social ranking.**

These results raise the possibility that stress-response might influence social fate by impacting on mesolimbic dopamine neurons. To test the causality between low VTA dopamine neuron activity and higher social rank, we assessed the impact of inhibiting this cell population on social status. We expressed Gi-coupled hM4D receptor, in VTA dopamine neurons, by injecting a Cre-dependent adeno-associated viral vector (AAV8-hSyn-DIO-hM4D(Gi)-mCherry) into the VTA of DATiCre mice (31) (VTA<sup>inhib</sup> mice, Figure 5A). Controls were Cre-negative littermates injected with an AAV8-hSyn-mCherry. At the site of injection, in the VTA, 73.0±3.5% of TH-positive neurons were also mCherry-positive whereas only 3.0±0.4% of transduced cells were TH-negative (Figure 5A, 5B and 5C). Upon clozapine-N-oxide (CNO) activation, hM4D hyperpolarizes neurons through a Gi protein mediated activation of inward-rectifying potassium channels (32). We confirmed that such approach can reduce significantly

dopamine neurons firing using patch-clamp *ex vivo* recordings and in anesthetized mice (Figure 3C-D). Of note, decreasing VTA dopamine neuron activity did not impact locomotor activity or motivation to press a lever for a food reward (Figures S7A and S7B). However, it promoted resilience to stress exposure as VTA<sup>inhib</sup> mice showed intact sociability levels after repeated social defeats in contrast to control mice (Figure S7C).

We first tested whether decreasing dopamine neuron activity could reshape an established hierarchy. We formed tetrads of one hM4D-expressing and three control mice. After stabilization of the hierarchy for 3 days, measured in tube-test, we injected CNO prior tube-test for 5 extra days (Figure 6A). During the 3 days of stabilization, control and hM4D mice showed similar average number of contest wins, and average rank attained (4.5 wins out of 9 contests per day and a rank of 2.5, consistent with the expected chance levels, Figure S8A-B)). Upon CNO treatment, VTA<sup>inhib</sup> mice progressively displayed a significant increase in the number of wins and rank attained, compared to controls. The difference did not show up at the first day of CNO injection, ruling out an acute effect of CNO on tube-test performance (Figure 6B, left panel). To examine whether decreasing activity of VTA dopamine neurons from the onset of mouse colony formation could shape the social fate of individuals, we administered continuous CNO treatment through drinking water (Figure 6C) to 6 tetrads. During the last three days of stabilization, we observed that VTA<sup>inhib</sup> mice tended to have a higher average of daily wins compared to controls, with a cumulated number of 112 wins out of 162 contests ending with three out of the six VTA<sup>inhib</sup> mice reaching rank 1 (Figure 6D).

## Discussion

Within a few days, genetically identical mice living in small groups of four individuals establish a social organization. The social hierarchy can be determined observing differential precedence in tube-test (14). The attained social rank is stable over month periods, with limited switch between ranks within a tetrad. These switches are rarely observed in the lowest and highest ranked individuals, but frequent for the intermediate ranks. Behavioral analyses usually present an important interindividual variability and social ranking might at least partly account for this phenomenon. We showed, in agreement with Larrieu *et al.* (15), that highest ranked mice exhibit higher anxiety-like behaviors and increased social interactions. Varholick *et al.* (33) did not however observe these correlations. This discrepancy may rely on the limited number of individuals tested or on the approach to identify ranking, with sparser tube-tests, performed once a week for three weeks. Other studies that used aggressiveness to identify dominant individuals led to conflicting results (34, 35). 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.* (36). This association of high anxiety and high sociability is surprising as in both humans and rodents, low anxiety is often paired with increased sociability (37, 6). For instance, oxytocin facilitates social behaviors and has well-known anxiolytic properties (38), and optogenetics stimulation of basolateral amygdala to ventral hippocampus circuit facilitates anxiety and impairs social interaction (39, 40). This associative rule is nevertheless not systematic, since vasopressin promotes social behavior and is anxiogenic (41).

A central but poorly explored question is whether the emergence of social ranks precedes the appearance of specific individual behavioral traits, or whether pre-existing individual traits channel the social status trajectory. Our study indicates that both situations occur. The difference in anxiety between highest and lowest ranked animals clearly emerged during social life, whereas the difference in sociability clearly pre-exists to the formation of ranks within colonies. Concerning anxiety, our observation is similar to what observed in outbred Swiss mice, housed in dyads and ranked upon their aggressiveness (42), however in rats a high level of anxiety is a predisposing factor for social submission (23). The origin of the higher sociability of future R1 individuals most likely arises from previous social housing conditions. It could have emerged in the previous colony in which these animals were grouped

before their arrival at the laboratory, or have happened before weaning, since a study suggested that maternal care could shape adult social behavior (43).

Although the literature is less abundant than for males, female colonies form social hierarchies that can be identified by tube-tests, may be more robust than in males (33) and do affect their behavioral responses to stress (44). One can expect that dopaminergic drive and GR would be in play in females, though it remains an open question.

The mesocorticolimbic system is a potential substrate for social ranking. In the NAcc, lower rank in dyadic contests is linked with low mitochondrial activity (23). In the PFC, higher social ranking is associated with higher strength of excitatory inputs (14), and dopaminergic neurons in this region play distinct roles depending on whether they express dopamine receptor 1 or 2, determining dominance and subordination, respectively (45). Our data shows that VTA dopamine neurons exhibit differential activity depending on the rank and that lowering dopamine activity can change the social trajectory of mice by promoting higher rank. Several studies suggested a link between dopamine and social ranking from insects to mammals (46). In ants, brain dopamine concentration is higher in socially dominant individuals (47, 48). In the striatal structure of birds and lizards, higher ranked individuals have increased levels of dopamine (49, 50). In line with our results, dominant rats have reduced levels of dopamine in the NAcc of (51).

Genetic evidence also sustains a link between dopaminergic neurotransmission and social status. Dopamine transporter gene inactivation in mouse disorganizes social colonies (52), and genetic variants in macaques are associated with social dominance (53). For dominant individuals, imaging in humans and non-human primates showed an enhanced availability of the striatal D2 receptor, that could indicate a reduced dopamine release (54, 55). Neuropharmacological approaches also suggest a role for dopamine signaling in social ranking although the differences in approaches (e.g. systemic vs. NAcc injections) do not allow clear interpretation. Systemic administration of D2-receptor antagonist reduced social dominance in both mice and monkeys (56) whereas injection into the NAcc of an agonist did not have an effect in rats (57). Similar experiments with a D1-receptor antagonist facilitated or did not modify social dominance in mice and monkeys (58) whereas injection into the NAcc of an agonist increased dominance in rats (57). Interestingly, changes in VTA dopamine cell activity are observed during the emergence of behavioral categories occurring within groups of dozens of mice living in complex semi-

naturalistic environments (13). It would be interesting to study social ranking between these categories using precedence tests such as the tube-test.

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 (26, 59). The lower cocaine sensitization of R1 individuals is coherent with their reduced dopamine neuron activity (60). For repeated social defeat, optogenetics stimulation of VTA neurons projecting to the NAcc induces a susceptible phenotype (19) whereas optogenetics (19) or chemogenetics inhibition (this study) induces resilience, in line with the increased resilience in R1 mice. Two studies made observations that differ from ours on the consequences of repeated social defeats depending on social rank. Lehmann *et al.* (61) did not observe a correlation whereas Larrieu *et al.* (15) observed more resilience for lower ranked individuals), LeClair *et al.* observed however effects similar to those we report (62). Differences between our data and that of Larrieu *et al.* may reside in the intensity of the defeats, the lower number of tetrads tested (8 here vs. 4-5), and the fact that we studied tetrads established for several months, regularly tested to ensure their stability over time.

Studies in human and animals suggested the existence of a link between social ranks and stress hormone differences. Elevated circulating glucocorticoids are usually associated with subordinate status in non-mammals and mammals, including rodents and primates, although conflicting results have been reported (8, 25, 9, 63). In human, higher socioeconomic status has been linked to lower evening glucocorticoid levels (64). Studies in military leaders, as well as in influential individuals from a Bolivian forager-farmer population showed lower glucocorticoid levels (65, 66). These findings are in line with the fact that mice deprived of GR signaling in dopamine-innervated regions exhibit higher social ranking when raised in tetrads or in dyads (67). These GR<sup>D1Cre</sup> mice also exhibit a lower VTA dopamine neuron activity (29), a decreased sensitization to cocaine (30), and are more resilient to repeated social defeat (20). These phenotypes are strikingly similar to that of R1 individuals suggesting that stress response and its impact on the dopamine pathway might play a principle organizational role in shaping the behavioral trajectories leading to the establishment of social ranking. Altogether, our results point dopamine as a key player in determining the social fate of individuals, a process that may occur under the continuous influence of the glucocorticoid stress-response.

## **Acknowledgments**

The authors warmly thank Soumee Bhattacharya, Carmen Sandi, Nicole Faucon, Rolando Meloni, Laurence Amar and Sheela Vyas for helpful discussions and S.B., L. A., N. F. and R. M. and S.V. for critical reading of the manuscript. The authors wish to thank Fabrice Machulka and the IBPS animal facilities. This work was supported by a Sorbonne Université grant (Emergence), the Labex BioPsy, the Foundation for Medical Research (FRM-Equipe grant DEQ20140329552 to FT and FRM DEQ20180339159 to JB), the INCA (TABAC-19\_020 grant to PF, JB and FT), and the Agence Nationale de la Recherche (ANR 3053NEUR31438301, ANR-17-CE37-0007, and ANR 21-CE14-0072 to FT; and ANR-14-CE35-0029-01 to SP). TC is a recipient fourth-year Ph.D. fellowship from the FRM (FDT202106012968). A preprint version of this work has been deposited on BioRxiv and HAL databases.

### **Author contributions**

DB, CV, CN, AZ, ACM, SM, AF, GD, FM, TC, JPT, TL, DR, EC, AL and SP performed experiments; FT, SP, DB, CV, JB and PF analyzed the data, FT and SP designed the study; FT, DB and SP wrote the manuscript, CV, FM, PF and JB reviewed and edited it.

### **Disclosures**

The authors declare no conflict of interest.

## References

1. Tinbergen N (1939): On the analysis of social organization among vertebrates, with special reference to birds. *Am Midl Nat* 21: 210.
2. Francis RC (1984): The effects of bidirectional selection for social dominance on agonistic behavior and sex ratios in the paradise fish (*Macropodus opercularis*). *Eff Bidirectional Sel Soc Domin Agonistic Behav Sex Ratios Paradise Fish Macropodus Opercularis* 90: 25–45.
3. Bergmüller R, Taborsky M (2010): Animal personality due to social niche specialisation. *Trends Ecol Evol* 25: 504–511.
4. Lathe R (2004): The individuality of mice. *Genes Brain Behav* 3: 317–327.
5. Berry RJ, Bronson FH (1992): Life history and bioeconomy of the house mouse. *Biol Rev Camb Philos Soc* 67: 519–550.
6. Beery AK, Kaufer D (2015): Stress, social behavior, and resilience: insights from rodents. *Neurobiol Stress* 1: 116–127.
7. Zhou T, Sandi C, Hu H (2018): Advances in understanding neural mechanisms of social dominance. *Curr Opin Neurobiol* 49: 99–107.
8. Sapolsky R (2004): Social Status and Health in Humans and Other Animals. *Annu Rev Anthr* 33: 393–418.
9. Creel S, Dantzer B, Goymann W, Rubenstein DR (2013): The ecology of stress: effects of the social environment ((R. Boonstra, editor)). *Funct Ecol* 27: 66–80.
10. Cordero MI, Sandi C (2007): Stress amplifies memory for social hierarchy. *Front Neurosci* 1: 175–184.
11. Timmer M, Sandi C (2010): A role for glucocorticoids in the long-term establishment of a social hierarchy. *Psychoneuroendocrinology* 35: 1543–1552.
12. Freund J, Brandmaier AM, Lewejohann L, Kirste I, Kritzler M, Krüger A, *et al.* (2013): Emergence of individuality in genetically identical mice. *Science* 340: 756–759.
13. Torquet N, Marti F, Campart C, Tolu S, Nguyen C, Oberto V, *et al.* (2018): Social interactions impact on the dopaminergic system and drive individuality. *Nat Commun* 9: 3081.
14. 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.
15. 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. *Curr Biol CB* 27: 2202-2210.e4.
16. Kessler RC, McGonagle KA, Zhao S, Nelson CB, Hughes M, Eshleman S, *et al.* (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.
17. Singh-Manoux A, Marmot MG, Adler NE (2005): Does subjective social status predict health and change in health status better than objective status? *Psychosom Med* 67: 855–861.
18. Gunaydin LA, Grosenick L, Finkelstein JC, Kauvar IV, Fenno LE, Adhikari A, *et al.* (2014): Natural neural projection dynamics underlying social behavior. *Cell* 157: 1535–1551.
19. Chaudhury D, Walsh JJ, Friedman AK, Juarez B, Ku SM, Koo JW, *et al.* (2013): Rapid regulation

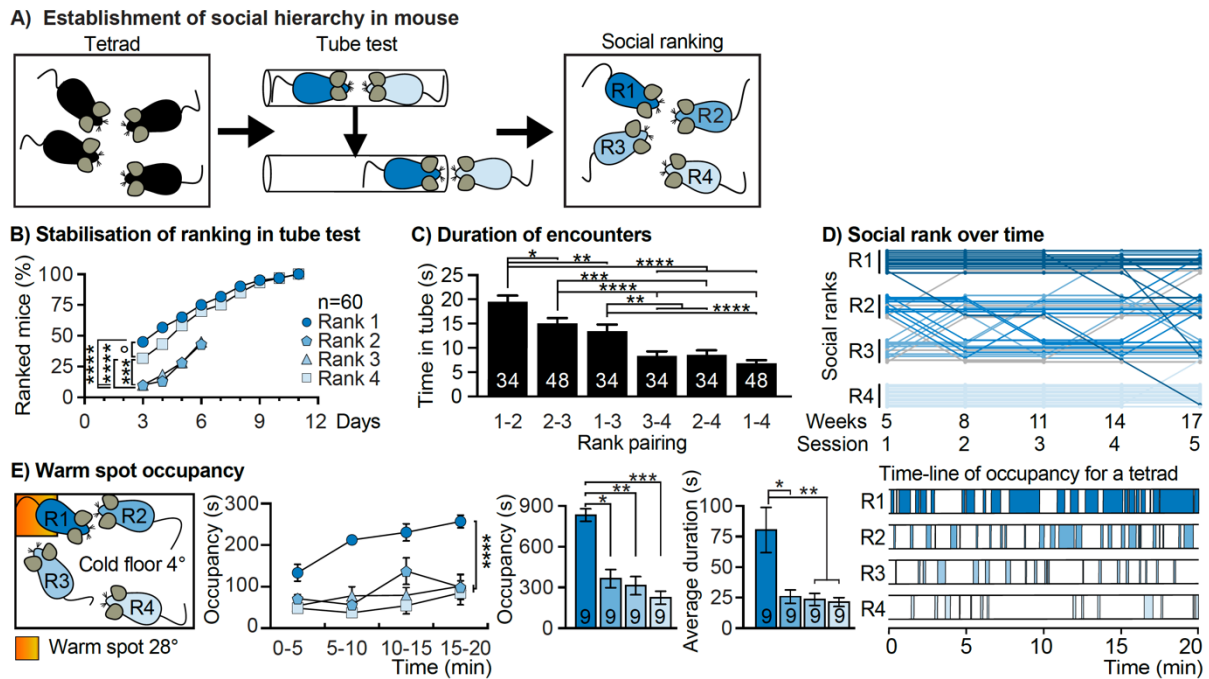


- of depression-related behaviours by control of midbrain dopamine neurons. *Nature* 493: 532–536.
20. Barik J, Marti F, Morel C, Fernandez SP, Lanteri C, Godeheu G, *et al.* (2013): Chronic stress triggers social aversion via glucocorticoid receptor in dopaminoceptive neurons. *Science* 339: 332–335.
  21. Zhou T, Zhu H, Fan Z, Wang F, Chen Y, Liang H, *et al.* (2017): History of winning remodels thalamo-PFC circuit to reinforce social dominance. *Science* 357: 162–168.
  22. Padilla-Coreano N, Batra K, Patarino M, Chen Z, Rock RR, Zhang R, *et al.* (2022): Cortical ensembles orchestrate social competition through hypothalamic outputs. *Nature* 603: 667–671.
  23. Hollis F, van der Kooij MA, Zanoletti O, Lozano L, Cantó C, Sandi C (2015): Mitochondrial function in the brain links anxiety with social subordination. *Proc Natl Acad Sci U S A* 112: 15486–15491.
  24. Stringhini S, Carmeli C, Jokela M, Avendaño M, Muennig P, Guida F, *et al.* (2017): Socioeconomic status and the 25 × 25 risk factors as determinants of premature mortality: a multicohort study and meta-analysis of 1·7 million men and women. *Lancet Lond Engl* 389: 1229–1237.
  25. Sapolsky RM (2005): The influence of social hierarchy on primate health. *Science* 308: 648–652.
  26. Robinson TE, Berridge KC (2000): The psychology and neurobiology of addiction: an incentive-sensitization view. *Addict Abingdon Engl* 95 Suppl 2: S91-117.
  27. Krishnan V, Han M-H, Graham DL, Berton O, Renthal W, Russo SJ, *et al.* (2007): Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell* 131: 391–404.
  28. Green MF (2016): Impact of cognitive and social cognitive impairment on functional outcomes in patients with schizophrenia. *J Clin Psychiatry* 77 Suppl 2: 8–11.
  29. Ambroggi F, Turiault M, Milet A, Deroche-Gamonet V, Parnaudeau S, Balado E, *et al.* (2009): Stress and addiction: glucocorticoid receptor in dopaminoceptive neurons facilitates cocaine seeking. *Nat Neurosci* 12: 247–249.
  30. Barik J, Parnaudeau S, Saint Amaux AL, Guiard BP, Golib Dzib JF, Bocquet O, *et al.* (2010): Glucocorticoid receptors in dopaminoceptive neurons, key for cocaine, are dispensable for molecular and behavioral morphine responses. *Biol Psychiatry* 68: 231–239.
  31. Turiault M, Parnaudeau S, Milet A, Parlato R, Rouzeau J-D, Lazar M, Tronche F (2007): Analysis of dopamine transporter gene expression pattern -- generation of DAT-iCre transgenic mice. *FEBS J* 274: 3568–3577.
  32. Armbruster BN, Li X, Pausch MH, Herlitze S, Roth BL (2007): Evolving the lock to fit the key to create a family of G protein-coupled receptors potently activated by an inert ligand. *Proc Natl Acad Sci U S A* 104: 5163–5168.
  33. Varholick JA, Bailoo JD, Palme R, Würbel H (2018): Phenotypic variability between Social Dominance Ranks in laboratory mice. *Sci Rep* 8: 6593.
  34. Hilakivi LA, Lister RG, Durcan MJ, Ota M, Eskay RL, Mefford I, Linnoila M (1989): Behavioral, hormonal and neurochemical characteristics of aggressive alpha-mice. *Brain Res* 502: 158–166.
  35. Ferrari PF, Palanza P, Parmigiani S, Rodgers RJ (1998): Interindividual variability in Swiss male mice: relationship between social factors, aggression, and anxiety. *Physiol Behav* 63: 821–827.
  36. Kunkel T, Wang H (2018): Socially dominant mice in C57BL6 background show increased social

- motivation. *Behav Brain Res* 336: 173–176.
37. Allsop SA, Vander Weele CM, Wichmann R, Tye KM (2014): Optogenetic insights on the relationship between anxiety-related behaviors and social deficits. *Front Behav Neurosci* 8: 241.
  38. Insel TR (2010): The challenge of translation in social neuroscience: a review of oxytocin, vasopressin, and affiliative behavior. *Neuron* 65: 768–779.
  39. Felix-Ortiz AC, Beyeler A, Seo C, Leppla CA, Wildes CP, Tye KM (2013): BLA to vHPC inputs modulate anxiety-related behaviors. *Neuron* 79: 658–664.
  40. Felix-Ortiz AC, Tye KM (2014): Amygdala inputs to the ventral hippocampus bidirectionally modulate social behavior. *J Neurosci Off J Soc Neurosci* 34: 586–595.
  41. Bielsky IF, Hu S-B, Szegda KL, Westphal H, Young LJ (2004): Profound impairment in social recognition and reduction in anxiety-like behavior in vasopressin V1a receptor knockout mice. *Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol* 29: 483–493.
  42. Hilakivi-Clarke LA, Lister RG (1992): Are there preexisting behavioral characteristics that predict the dominant status of male NIH Swiss mice (*Mus musculus*)? *J Comp Psychol Wash DC* 1983 106: 184–189.
  43. Starr-Phillips EJ, Beery AK (2014): Natural variation in maternal care shapes adult social behavior in rats. *Dev Psychobiol* 56: 1017–1026.
  44. Smith-Osborne L, Duong A, Resendez A, Palme R, Fadok JP (2023): Female dominance hierarchies influence responses to psychosocial stressors. *Curr Biol CB* 33: 1535-1549.e5.
  45. Xing B, Mack NR, Zhang Y-X, McEachern EP, Gao W-J (2022): Distinct Roles for Prefrontal Dopamine D1 and D2 Neurons in Social Hierarchy. *J Neurosci Off J Soc Neurosci* 42: 313–324.
  46. Yamaguchi Y, Lee Y-A, Goto Y (2015): Dopamine in socioecological and evolutionary perspectives: implications for psychiatric disorders. *Front Neurosci* 9: 219.
  47. Penick CA, Brent CS, Dolezal K, Liebig J (2014): Neurohormonal changes associated with ritualized combat and the formation of a reproductive hierarchy in the ant *Harpegnathos saltator*. *J Exp Biol* 217: 1496–1503.
  48. 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. *J Exp Biol* 218: 1091–1098.
  49. McIntyre DC, Chew GL (1983): Relation between social rank, submissive behavior, and brain catecholamine levels in ring-necked pheasants (*Phasianus colchicus*). *Behav Neurosci* 97: 595–601.
  50. Korzan WJ, Forster GL, Watt MJ, Summers CH (2006): Dopaminergic activity modulation via aggression, status, and a visual social signal. *Behav Neurosci* 120: 93–102.
  51. Jupp B, Murray JE, Jordan ER, Xia J, Fluharty M, Shrestha S, *et al.* (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.
  52. Rodriguiz RM, Chu R, Caron MG, Wetsel WC (2004): Aberrant responses in social interaction of dopamine transporter knockout mice. *Behav Brain Res* 148: 185–198.
  53. Miller-Butterworth CM, Kaplan JR, Shaffer J, Devlin B, Manuck SB, Ferrell RE (2008): Sequence

- variation in the primate dopamine transporter gene and its relationship to social dominance. *Mol Biol Evol* 25: 18–28.
54. Nader MA, Nader SH, Czoty PW, Riddick NV, Gage HD, Gould RW, *et al.* (2012): Social dominance in female monkeys: dopamine receptor function and cocaine reinforcement. *Biol Psychiatry* 72: 414–421.
  55. Martinez D, Orłowska D, Narendran R, Slifstein M, Liu F, Kumar D, *et al.* (2010): Dopamine type 2/3 receptor availability in the striatum and social status in human volunteers. *Biol Psychiatry* 67: 275–278.
  56. 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. *Sci Rep* 7: 43348.
  57. van der Kooij MA, Hollis F, Lozano L, Zalachoras I, Abad S, Zanoletti O, *et al.* (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.
  58. Yamaguchi Y, Lee Y-A, Kato A, Goto Y (2017): The Roles of Dopamine D1 Receptor on the Social Hierarchy of Rodents and Nonhuman Primates. *Int J Neuropsychopharmacol* 20: 324–335.
  59. Russo SJ, Murrough JW, Han M-H, Charney DS, Nestler EJ (2012): Neurobiology of resilience. *Nat Neurosci* 15: 1475–1484.
  60. Runegaard AH, Sørensen AT, Fitzpatrick CM, Jørgensen SH, Petersen AV, Hansen NW, *et al.* (2018): Locomotor- and Reward-Enhancing Effects of Cocaine Are Differentially Regulated by Chemogenetic Stimulation of Gi-Signaling in Dopaminergic Neurons. *eNeuro* 5: ENEURO.0345-17.2018.
  61. Lehmann ML, Geddes CE, Lee JL, 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: e69822.
  62. LeClair KB, Chan KL, Kaster MP, Parise LF, Burnett CJ, Russo SJ (2021): Individual history of winning and hierarchy landscape influence stress susceptibility in mice. *eLife* 10: e71401.
  63. Cavigelli SA, Caruso MJ (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: 20140103.
  64. Cohen S, Schwartz JE, 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.
  65. Sherman GD, Lee JJ, Cuddy AJC, Renshon J, Oveis C, Gross JJ, Lerner JS (2012): Leadership is associated with lower levels of stress. *Proc Natl Acad Sci U S A* 109: 17903–17907.
  66. von Rueden CR, Trumble BC, Emery Thompson M, Stieglitz J, Hooper PL, Blackwell AD, *et al.* (2014): Political influence associates with cortisol and health among egalitarian forager-farmers. *Evol Med Public Health* 2014: 122–133.
  67. Papilloud A, Weger M, Bacq A, Zalachoras I, Hollis F, Larriou T, *et al.* (2020): The glucocorticoid receptor in the nucleus accumbens plays a crucial role in social rank attainment in rodents. *Psychoneuroendocrinology* 112: 104538.

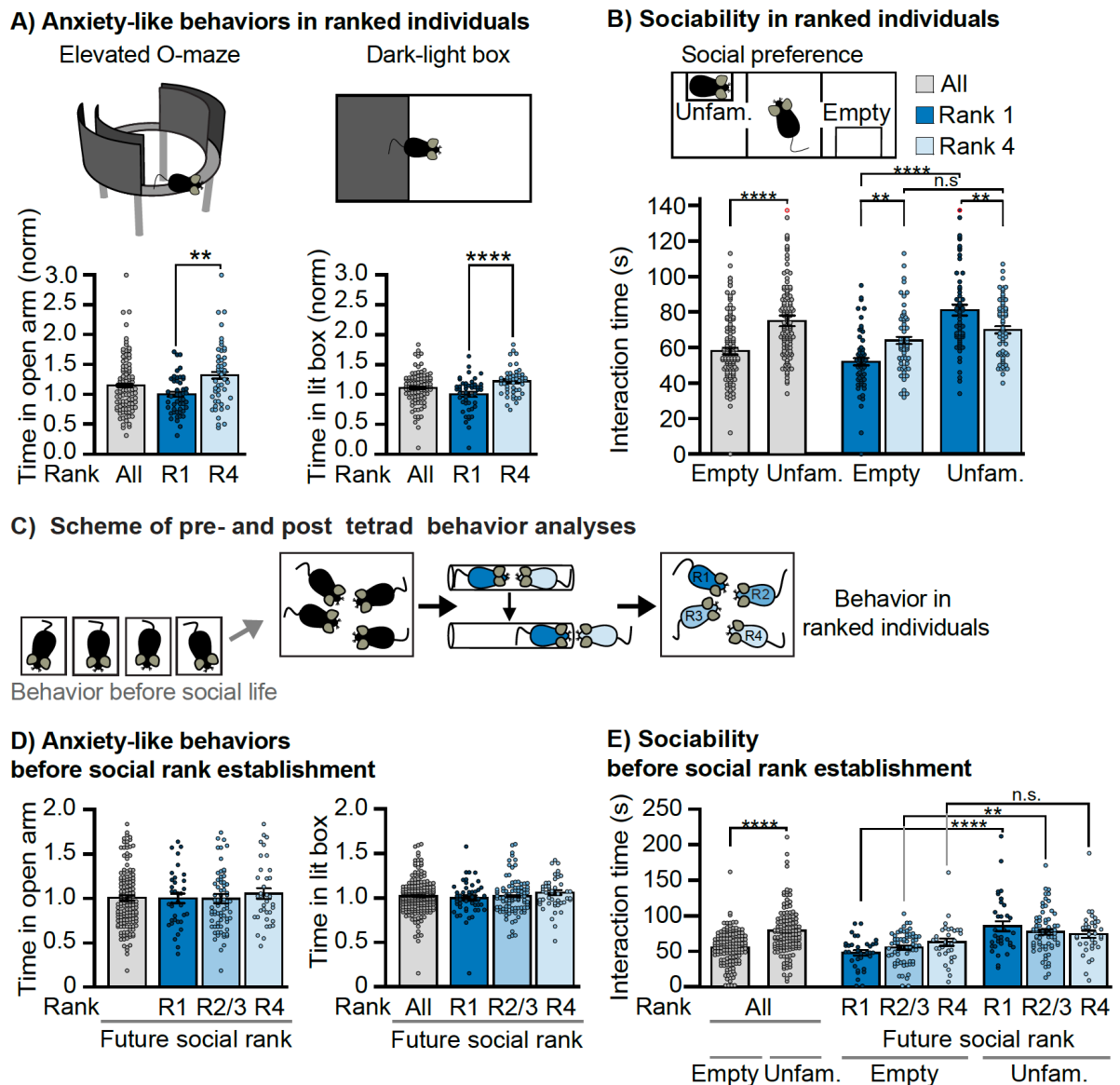
## Figures



**Figure 1. Social hierarchy establishment and stability in mice.**

**(A)** Design of social hierarchy establishment and analysis. Unfamiliar male mice were grouped into four. After 2 to 4 weeks, their social rank was determined by a precedence test (tube-test) and further tested four times until week 17. **(B)** Rank identification in the tube-test. The cumulated percentage of stable ranked individuals for each rank is shown for each day of the tube-test ( $n=60$  tetrads). The data for ranks 2 and 3 are indicated for days 3 to 6 since rank identification was stopped when ranks 1 and 4 were stable. Gehan-Breslow-Wilcoxon test; R1 vs. R4  $p=0.08$ ; R1 vs. R2-R3 and R4 vs. R2-R3  $****p<0.0001$ . **(C)** Mean duration of the confrontation in the tube-test performed during the three last days when ranks were stable. Each possible rank combination is pictured. The numbers of tetrads that have been analyzed are indicated for each rank combination;  $n=48$  or  $34$  in cases of unstable ranks 2 and 3. Wilcoxon rank-sum test  $*p<0.05$ ;  $**p<0.01$ ;  $***p<0.001$ ;  $****p<0.0001$ . Error bars,  $\pm$  SEM. **(D)** Social ranks were stable from week 5 and for over three months for most animals. The dynamic of social ranking in the tube-test is pictured for a set of 12 tetrads. Each line depicts an individual mouse, its position within its social rank pool indicates the tetrad to which it belongs. Different blue intensities indicate the rank defined at the first tube-test session. The 6 individuals in ranks 2 and 3 that did not reach stability at the end of the first session are pictured with grey lines. These results are detailed day by day in Figure S2A. **(E)** Left: representation of the warm spot test. The position of the warm spot is pictured by an orange box. Middle panel, left chart: time course occupancy of the warm spot. Main social rank effect,  $F(3, 32)=22.08$ ;  $****p<0.0001$ . Main time effect,  $F(3, 96)=11.06$ ;  $p<0.0001$ . Interaction social rank  $\times$  time  $F(9, 96)=2.52$ ;  $p<0.05$ . Two-way repeated ANOVA followed by Bonferroni correction R1 vs. R2; R1 vs. R3; R1 vs. R4;  $p<0.0001$ . Middle panel, middle chart: total occupancy of the warm spot. Main social rank effect,  $p<0.001$ . Kruskal-Wallis non-parametric test followed by Dunn correction R1 vs. R2  $*p<0.05$ ; R1 vs. R3  $**p<0.01$ ; R1 vs. R4  $***p<0.001$ . Middle panel, right chart:

average duration of occupancy by differently ranked individuals. Main social rank effect  $p < 0.001$ . Kruskal-Wallis non-parametric test followed by Dunn correction R1 vs. R2  $*p < 0.05$ ; R1 vs. R3 and R1 vs. R4  $**p < 0.01$ .  $n = 9$  tetrads; error bars,  $\pm$  SEM. Right panel: representative occupancy periods of the warm spot by individuals of a tetrad.



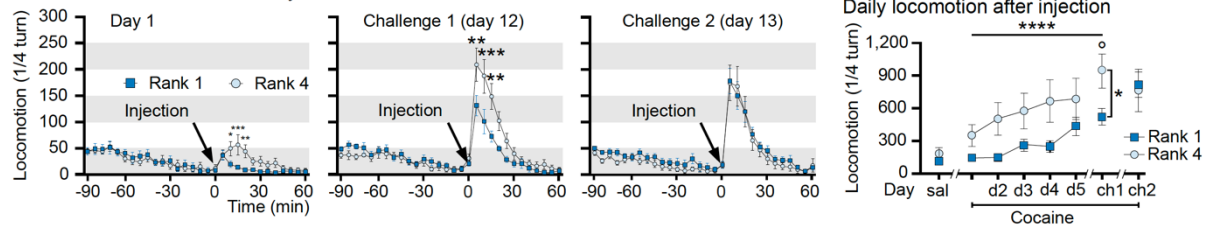
**Figure 2. Differences in sociability between highest and lowest-ranked individuals preexist to rank establishment whereas anxiety emerges from social life within tetrads.**

**(A)** Rank 1 individuals display increased anxiety-like behaviors. The experimental setups are pictured. The time spent for all C57B/L6 ( $n=96$ ), Rank 1 (R1,  $n=48$ , dark blue), and Rank 4 (R4,  $n=48$ , light blue) individuals in the open-section of an elevated O-maze and lit compartment of a dark-light box are pictured. Time values were normalized from the R1 means. Two-tailed, Mann Whitney test  $**p<0.01$ ;  $****p<0.0001$ . Error bars,  $\pm$  SEM. **(B)** Highest ranked mice display increased sociability. The three-chamber test is depicted. The duration of time spent interacting with an empty box (Empty) and with a box containing an unfamiliar mouse (Unfam.) is pictured for all C57B/L6 mice (grey,  $n=115$ ), R1 (dark blue,  $n=57$ ) and R4 (light blue,  $n=58$ ) individuals. All mice: Wilcoxon matched-pairs signed rank test,  $****p<0.0001$ . Right graph: social cue effect,  $F(1, 113)=41.7$ ;  $p<0.0001$ . No effect of social rank  $F(1,113)=0.002$ ;  $p=0.97$ . Interaction social cue  $\times$  rank,  $F(1,113)=17.07$ ;  $p<0.0001$ . Two-way repeated ANOVA followed by Bonferroni correction, R1 empty vs. unfam  $****p<0.0001$ ; R1 vs. R4  $**p<0.01$ . Error

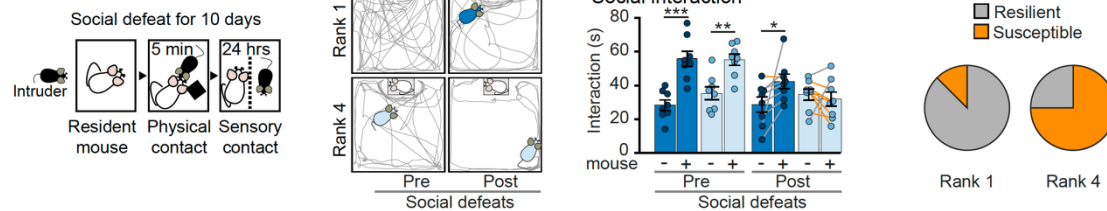
bars,  $\pm$  SEM. **(C)** Before being grouped in tetrads, behavior was assessed in singly housed individuals. **(D)** Before rank establishment, future R1 and future R4 individuals display similar anxiety-like behaviors. The time spent exploring the open segments of an elevated O-maze and the lit compartment of a dark-light box are pictured for all C57BL/6 mice (grey bars,  $n=144$  and  $n=192$ , respectively), and among them the future rank 1 (dark blue bars,  $n=36$  and  $n=48$ , respectively), the future ranks 2 and 3 (medium blue bars,  $n=72$  and  $n=95$ , respectively), and the future R4 (light blue bars,  $n=36$  and  $n=48$ , respectively). Kruskal-Wallis non-parametric test,  $p=0.30$  and  $p=0.62$ , respectively. Error bars,  $\pm$  SEM. **(E)** Future rank 4 mice did not display social preference, unlike future rank 1 individuals. Duration of interactions with an empty box vs a box containing an unfamiliar (Unfam.) congener is shown for 136 mice C57BL/6 mice from tetrads (grey bars) and, among them, the future rank 1 (dark blue bars,  $n=34$ ), the future ranks 2/3 (medium blue bars,  $n=68$ ) and the future rank 4 (light blue bars,  $n=34$ ). All mice,  $p<0.0001$ , Wilcoxon matched-pairs signed rank test, \*\*\*\* $p<0.0001$ . Right graph: Main social cue effect,  $F(1, 133)=27.78$ ;  $p<0.0001$ . No effect of social rank  $F(2,133)=0.243$ ;  $p=0.78$ . No interaction, social cue x rank  $F(2,113)=2.41$ ;  $p<0.0001$ . Two-way repeated ANOVA followed by Bonferroni correction, R1 Empty vs. Unfam \*\*\*\* $p<0.0001$ ; R2/3 Empty vs. Unfam \*\* $p<0.01$ . Error bars,  $\pm$  SEM.

### A) Locomotor sensitization to cocaine

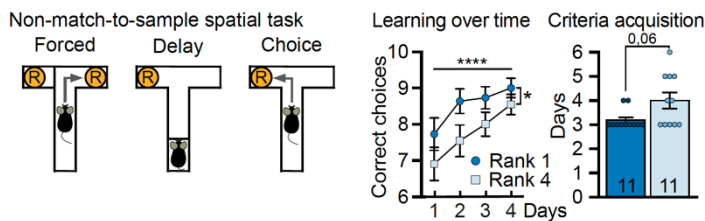
Time course of locomotor activity



### B) Repeated social defeat



### C) Spatial working memory



**Figure 3. Social rank correlates with differences in preclinical models of behavioral disorders.**

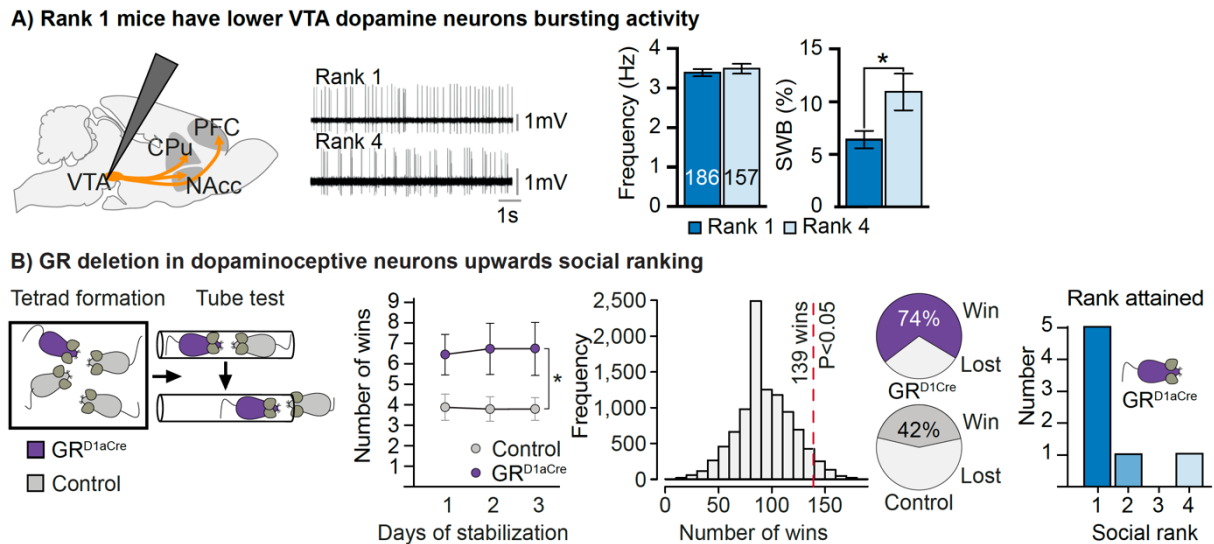
**(A)** Locomotor sensitization to cocaine. Left, time course of Rank 1 ( $n=10$ ) and Rank 4 ( $n=9$ ) individuals locomotion expressed as  $\frac{1}{4}$  turn within a circular corridor for indicated sessions. Time 0 corresponds to the injection of cocaine ( $10 \text{ mg kg}^{-1}$ ), except for Challenge 2 (ch2) for which R4 individuals received  $10 \text{ mg kg}^{-1}$  and R1  $12.5 \text{ mg kg}^{-1}$ . For day 1, challenge 1 and challenge 2, two-way ANOVA, followed by Bonferroni correction R1 vs. R4  $*p<0.05$ ;  $**p<0.01$ ;  $***p<0.001$ . Right panel, 1 hour cumulated locomotor activity of R1 and R4 individuals (dark and light blue, respectively) following saline (sal), or cocaine (day 1 to 5, and challenge 1 and 2). For day 1 to Challenge 1: main time effect,  $F(5, 85)=13.53$ ,  $**** p<0.0001$ . Main social rank effect,  $F(1, 17)=5.34$ ,  $*p<0.05$ . Two-way repeated ANOVA followed by Bonferroni correction, challenge 1 R1 vs R4  $^{\circ}p<0.1$ . For Challenge 2, Mann-Whitney test,  $p=0.67$

**(B)** Depression-like behavior induced by repeated social defeats. Left, protocol design. Middle left, representation of the open field in which social interactions were measured. The position of the box containing a CD1 mouse is indicated. Representative trajectories of R1 and R4 individuals before and after repeated social defeats are pictured. Middle right panel, R1 (dark blue,  $n=8$ ) and R4 (light blue,  $n=8$ ) interaction time with an empty box (mouse -) or a CD1 mouse (mouse +), before and after repeated social defeat. Right panel, susceptible individuals, developing social aversion are indicated with orange lines, resilient ones with grey ones. Right, representation of susceptible (orange) and resilient (grey) individuals among R1 and R4 males. Pre-social defeats: effect of social cue  $F(1, 14)=41.2$ ,  $p<0.0001$ . No effect of social rank  $F(1, 14)=0.76$ ,  $p=0.40$ . No interaction  $F(1, 14)=1.04$ ,  $p=0.33$ . Two-way repeated ANOVA followed by Bonferroni correction, empty box vs social cue for R1 mice  $*** p<0.001$ ; empty box vs. social cue for R4 mice,  $**p<0.01$ . Post-social defeats: no effect of social cue  $F(1, 14)=1.89$ ,  $p=0.19$ . No effect of social



rank  $F(1, 14)=0.29$ ,  $p=0.60$ . effect of interaction  $F(1, 14)=4.95$ ,  $*p<0.05$ . Two-way repeated ANOVA followed by Bonferroni correction, empty box vs. social cue for R1 mice,  $*p<0.05$ . Error bars,  $\pm$  SEM.

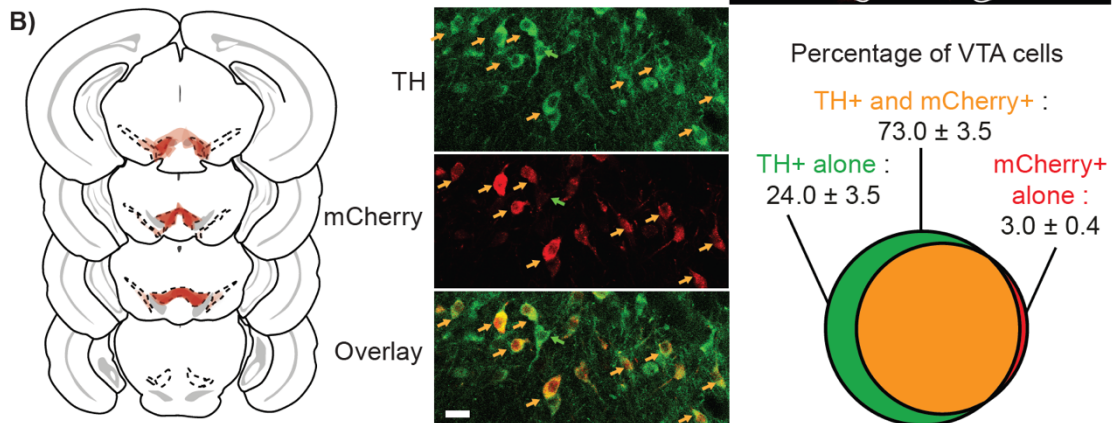
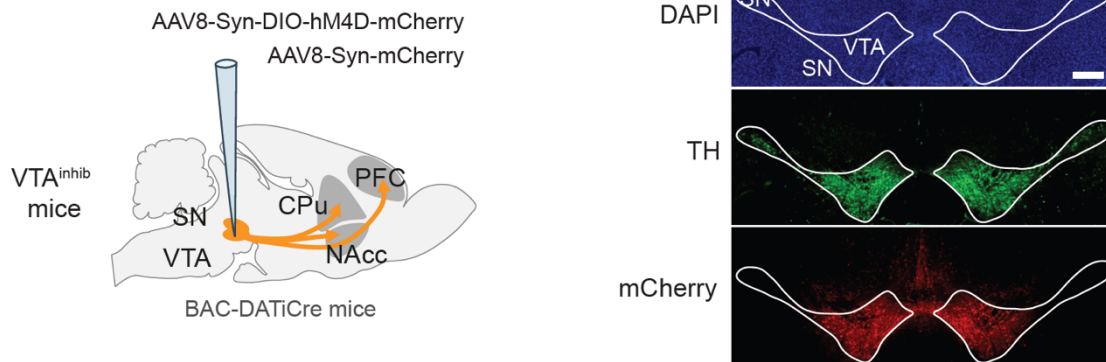
**(C)** Rank 1 individuals display better performances in the Non-Match-to-Sample-spatial task, a spatial working memory task. Left panel illustrates the task design. The learning curve of 11 mice from both ranks, indicates the progression of correct choices over the days (left chart). Effect of time  $F(3, 60)=7.87$ ,  $****p<0.0001$ . Effect of social rank  $F(1, 20)=4.85$ ,  $*P<0.05$ . No effect of interaction  $F(3, 60)=0.36$ ,  $p=0.78$ . Two-way repeated ANOVA. The Right chart 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,  $\pm$  SEM.



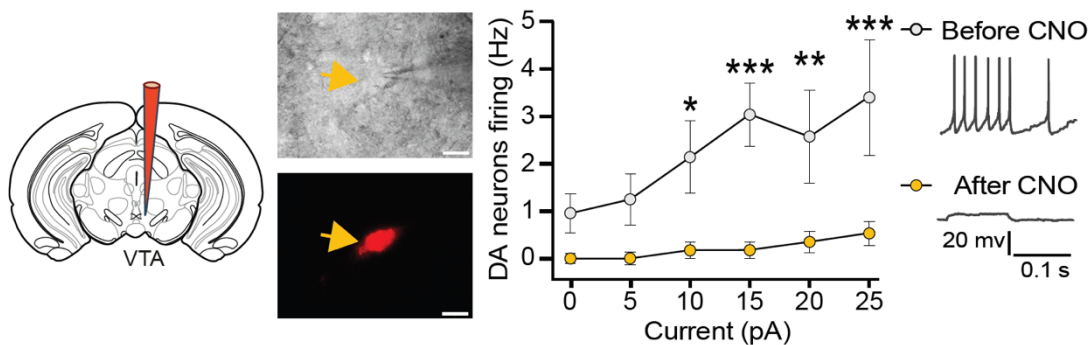
**Figure 4. Dopamine neuron activity in the ventral tegmental area and gene Glucocorticoid Receptor in dopaminergic neurons modulate social rank attainment.**

**(A)** Rank 1 individuals have lower VTA dopamine neuron bursting activity. Schematic representation of electrode positioning (left). Representative traces of recording for individuals of each rank (middle). 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) (right). Rank 1 %SWB vs. Rank 4 %SWB: \*p<0.016 ( $\Delta < \%SWB >_o \geq 4.5\%$ , n=163 on 10000 simulations). **(B)** GR gene deletion in dopaminergic neurons promotes a higher social ranking in tetrads. Tetrads were constituted with one mutant (GR<sup>D1aCre</sup>) and three control (GR<sup>loxP/loxP</sup>) mice, for three weeks before tube-test. Number of tetrads = 7 (GR<sup>loxP/loxP</sup> n=21 and GR<sup>D1aCre</sup> n=7). Left chart: average number of daily wins during the three days of rank stabilization. Main group effect F(1, 26)=5.51, \*p<0.05. Two-way repeated ANOVA. Error bars,  $\pm$  SEM. Middle left chart: over the three days of rank stabilization, GR<sup>D1aCre</sup> mice won a number of 139 cumulated contests out of 189. Histogram of the simulated number of wins obtained for a mouse chosen randomly in an ordered tetrad. Chance level, wins  $\geq 139$ , \*p<0.05 determined on 10000 simulations of 27 contests for 7 tetrad. Middle right: level of chance to win a contest for GR<sup>D1aCre</sup> and control individuals. Right chart: rank of each GR<sup>D1aCre</sup> mouse at the end of the stabilization. Chance level, \*p<0.05 on 10000 simulations.

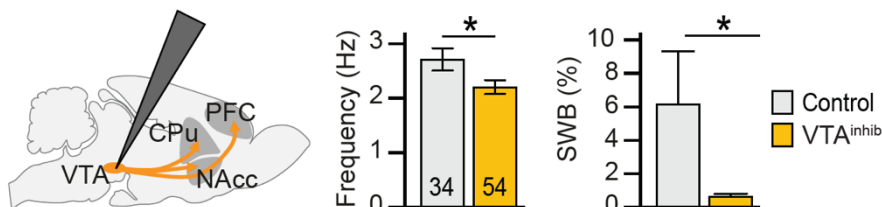
**A) DREADD approach in VTA dopamine neurons**



**C) Ex vivo DREADD mediated VTA dopamine neurons inhibition by CNO**



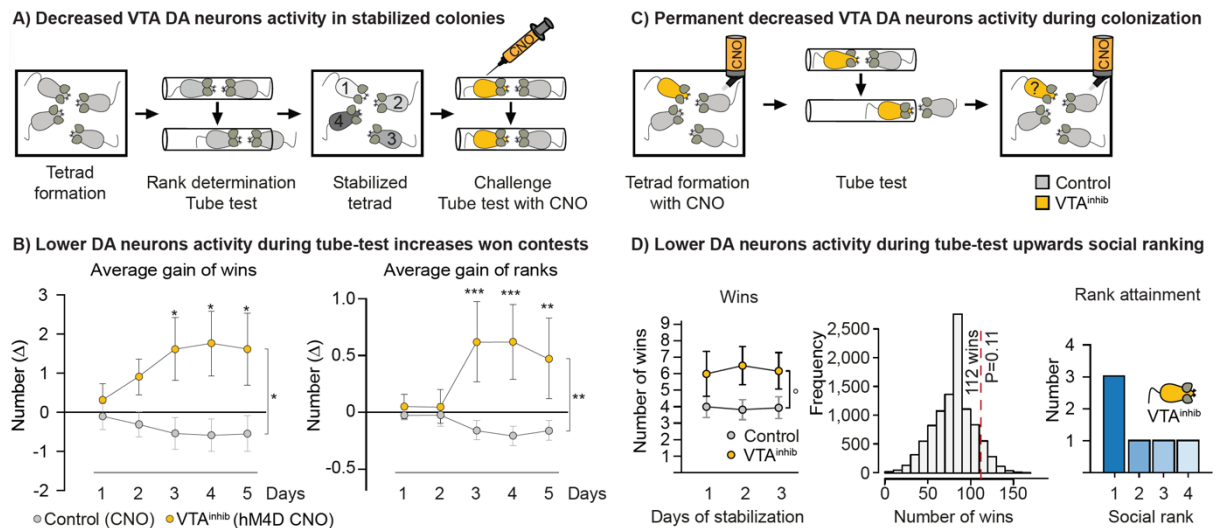
**D) In vivo DREADD mediated VTA dopamine neurons inhibition by CNO**



**Figure 5. DREADD-mediated VTA dopamine neuron inhibition.**

(A) Upper part, left panel: injection and expression of the hM4D in the VTA of BAC-DATiCre mice. Right panel: Representative image of injection site. Upper photo, DAPI staining, VTA: ventral tegmental area, SN: substantia nigra. Scale bar: 250  $\mu$ m. Middle photo, immunofluorescence staining detecting Tyrosine Hydroxylase (TH) expression. Lower photo, immunofluorescence staining detecting mCherry

expression. Lower part, Histology analysis of hM4D expression within the VTA. **(B)** Left: scheme of hM4D expression spreading (n=7). Middle panel: colocalization of mCherry (in red) with TH (in green) in the VTA. Yellow arrows point to neurons co-expressing TH and mCherry. The green arrow points to a neuron expressing only TH. Scale bar: 50  $\mu\text{m}$ . Right panel: quantification of neurons co-expressing TH and mCherry (orange), TH alone (green) or mCherry alone (red). **(C)** *Ex vivo* whole-cell patch clamp recordings in brain slices Four weeks after expression whole-cell patch clamp recordings were performed in brain slices. Upper left panel: schematic picturing of recording position; representative images showing dopamine cell expressing mCherry in *ex vivo* recordings. Scale bar = 20  $\mu\text{m}$ . Middle panel: bath application of CNO (30  $\mu\text{M}$ ) during 10 min blocks dopamine firing induced by increasing current injections. n=7 from 4 individuals. Interaction treatment  $\times$  current  $F(5, 30) = 1.620$ ,  $p=0.1850$ . Two-way repeated ANOVA followed by Bonferroni correction \* $p<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$ . Right panel: representative voltage traces for one neuron injected with 25pA before and after CNO application were shown. Error bars,  $\pm$  SEM. **(D)** *In vivo*, selective chemogenetic inhibition prevents dopamine neurons firing in brain slices. CNO treatment (10 mg  $\text{L}^{-1}$  during 10 to 15 days) in drinking water significantly reduced spontaneous firing frequency, and almost abolished the bursting activity of VTA dopamine neurons in anesthetized  $\text{VTA}^{\text{inhib}}$  mice when compared to control mice (Figure 5D).  $\text{VTA}^{\text{inhib}}$  : n=54 cells, 3 individuals; or mCherry expressing controls : n=34 cells, 3 individuals. Mean frequency (Hz) (left panel), \* $p<0.05$  Mann-Whitney test; and percentage of spikes within bursts (SWB) (right panel) DREADD %SWB vs. GFP %SWB: \* $P<0.035$  ( $\Delta\langle\%SWB\rangle_0 \geq 5.5\%$ , n=355 on 10000 simulations). Error bars,  $\pm$  SEM.



**Figure 6 Decreasing dopamine neurons activity promotes winning and hierarchy climbing.**

**(A)** Experimental design for decreasing VTA dopamine neuron activity in stabilized colonies. **(B)** Left panel: CNO treatment over 5 consecutive days leads to an increase in the number of wins in  $VTA^{inh}$  mice ( $n=7$ ) compared to controls ( $n=21$ ). Data are expressed as a difference score compared to the baseline (average number of wins during the 3 days of stabilization prior CNO treatment) (see Figure S8). Main group effect,  $F(1, 26)=6.68$ ,  $*p<0.05$ ; no time effect,  $F(4, 104)=0.63$ ,  $p=0.64$ ; interaction effect,  $F(4, 104)=2.54$ ;  $p<0.05$ . Two-way repeated ANOVA followed by Bonferroni correction,  $VTA^{inh}$  vs. control  $*p<0.05$ . Right panel: CNO treatment leads to an increase in rank in  $VTA^{inh}$  mice compared to controls. Main group effect,  $F(1, 26)=11.33$ ,  $**p<0.01$ ; no time effect,  $F(4, 104)=1.65$ ,  $p=0.17$ ; interaction effect,  $F(4, 104)=5.59$ ;  $p<0.001$ . Two-way repeated ANOVA followed by Bonferroni correction,  $VTA^{inh}$  vs control  $**p<0.01$ ;  $***p<0.001$ . Error bars,  $\pm$  SEM. **(C)** Experimental design for decreasing VTA dopamine neuron activity at the beginning of the social life in colony. **(D)** Left panel: average number of daily wins during the three days of hierarchy stabilization in  $VTA^{inh}$  ( $n=6$ ) and control mice ( $n=18$ ). Main group effect,  $F(1, 22)=3.31$ ,  $^{\circ}p=0.08$ ; no time effect,  $F(2, 44)=0.16$ ,  $p=0.85$ . Two-way repeated ANOVA. Error bars,  $\pm$  SEM. Middle chart: over the three days of rank stabilization,  $VTA^{inh}$  mice won a number of 112 cumulated contests out of 162. A histogram of the simulated number of wins obtained for a mouse choose randomly in an ordered tetrad. Chance level, wins  $\geq 112$ ,  $p=0.11$  determined on 10000 simulations of 27 contests for 6 tetrad. Right chart: rank of each  $VTA^{inh}$  mouse at the end of the stabilization. Chance level,  $p=0.17$  on 10000 simulations.

## Supplementary information

### Dopamine neurons activity and stress signalling as links between social hierarchy and psychopathology vulnerability.

Dorian Battivelli<sup>1,2,3</sup>, Cécile Vernochet<sup>1,2,3</sup>, Estelle Conabady<sup>1,2,3</sup>, Claire Nguyen<sup>2,3,4</sup>, Abdallah Zayed<sup>1,2,3</sup>, Ashley Lebel<sup>1,2,3</sup>, Aura Carole Meirsman<sup>1,2,3</sup>, Sarah Messaoudene<sup>1,2,3</sup>, Alexandre Fieggen<sup>1,2,3</sup>, Gautier Dreux<sup>1,2,3</sup>, Daiana Rigoni<sup>5,6</sup>, Tinaïg Le Borgne<sup>2,3,4</sup>, Fabio Marti<sup>2,3,4</sup>, Thomas Contesse<sup>5,6</sup>, Jacques Barik<sup>5,6</sup>, Jean-Pol Tassin<sup>1,2,3</sup>, Philippe Faure<sup>2,3,4</sup>, Sébastien Parnaudeau<sup>1,2,3,\*</sup>, †, François Tronche<sup>1,2,3,\*</sup>, †

<sup>1</sup> Gene Regulation and Adaptive Behaviours group, Neuroscience Paris Seine, UMR8246, Centre National de la Recherche Scientifique (CNRS), Institut de Biologie Paris Seine, 75005, Paris, France.

<sup>2</sup> INSERM U1130.

<sup>3</sup> Sorbonne Université UMCR18,

<sup>4</sup> Neurophysiology and Behaviour group, Neuroscience Paris Seine, UMR8246, CNRS.

<sup>5</sup> Université Côte d'Azur, Nice, France

<sup>6</sup> Institut de Pharmacologie Moléculaire & Cellulaire, CNRS UMR7275, Valbonne, France.

\* Corresponding authors: François Tronche ([francois.tronche@sorbonne-universite.fr](mailto:francois.tronche@sorbonne-universite.fr)) and Sébastien Parnaudeau ([sebastien.parnaudeau@sorbonne-universite.fr](mailto:sebastien.parnaudeau@sorbonne-universite.fr))

† These authors contributed equally to this work.

#### This PDF file includes:

Supplementary text

Figures S1 to S8

SI References

## Supplementary Information Text

### Material and Methods.

#### Animals

C57BL/6JRj, 129/SvEv, and CD1 male mice by 6 weeks were purchased from Janvier (Le Genest-Saint-Isle, France). Animals were weaned at three weeks of age, then housed by five in cages until shipment. Once received, they were housed as described below, 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. BAC-DATiCre<sup>ffto</sup> mice (1) were heterozygous and backcrossed on a C57BL/6J background. *Nr3c1* (*GR*) gene inactivation was selectively targeted in dopaminergic neurons (*Nr3c1*<sup>loxP/loxP</sup>;Tg:D1aCre (2), hereafter designed GR<sup>D1aCre</sup>), as described in Barik et al. *et al.* (20). Experimental animals were obtained by mating *Nr3c1*<sup>loxP/loxP</sup> females with *Nr3c1*<sup>loxP/loxP</sup>;Tg:D1aCre males. Half of the progeny was mutant animals whereas the other half was control littermates. Experiments were performed following the European Directive 2010/63/UE and the recommendation 2007/526/EC for the care of laboratory animals and approved by the Sorbonne Université committee for animal care and use.

#### Stereotaxic injections

Stereotaxic injections were performed using a stereotaxic frame (Kopf Instruments) under general anesthesia with xylazine and ketamine (10 mg kg<sup>-1</sup> and 150 mg kg<sup>-1</sup>, respectively). Anatomical coordinates and maps were adjusted from Watson and Paxinos, 2010 (3). The injection rate was set at 100 nl min<sup>-1</sup>. To express hM4D in VTA dopamine neurons, we injected 6 weeks old BAC-DATiCre<sup>ffto</sup> mice (3) with AAV8-hSyn-DIO-hM4D-mCherry (300 nl L<sup>-1</sup>, titration 10<sup>12</sup> particles ml<sup>-1</sup>, Addgene, Cat: 44362-AAV8) bilaterally in the VTA (AP -3.2 mm, ML ±0.6 mm, DV -4.7mm from the bregma). For controls, C57BL/6J mice issued from the same breeding as BAC-DATiCre<sup>ffto</sup> mice, but not carrying the transgene, were injected similarly but with an AAV8-hSyn-mCherry (titration 10<sup>12</sup> particles ml<sup>-1</sup>, Addgene, Cat: 114472-AAV8). Animals were given a 4 weeks recovery period to allow sufficient viral expression.

#### Constitution of tetrads

Mice were weighed and were then grouped by four (tetrads) gathering mice of similar weights, upon arrival at 6 weeks of age, or at 7-8 weeks of age when behavioral testing was performed before the constitution of the tetrads (see Figure S1). In that case, mice were single-housed for 7 to 10 days, including 2-4 days of habituation followed by behavioral testing. The tetrads were constituted right after. For the different tetrads studied, the timing of the behavioral studies performed is depicted in Figure S1. For tetrads including GR<sup>D1aCre</sup> mutant and BAC-DATiCre<sup>ffto</sup> mice. Tetrads were formed by animals unfamiliar to each other issued from different litters, grouping one mutant with three age-matched control mice (GR<sup>loxP/loxP</sup>).

#### Social rank identification

**Tube-test.** Mice gathered in groups of four individuals for two to four weeks were first trained to move forward in a transparent Plexiglas tube (diameter, 2.5 cm; length, 30 cm) for 2 consecutive days, performing 8 trials on the first day and 4 the second one. Each individual alternatively entered the tube from the right and left extremities and was let for a maximum of 30 seconds to exit the tube at the opposite end. After 30 seconds if still present within the tube, the mouse was gently pushed out. The diameter of the tube allowed passing one individual but 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 daily for each pairwise 3 trial sessions composed of 3 confrontations each (9 daily 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 described as the loser 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). Contests exceeding 2.5 minutes were stopped and immediately repeated. After each trial, the tube was cleaned with 20% ethanol and dried. Among more than 100 tetrads analyzed, we 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 further analyzed, until reaching three days of stability for ranks 1 and 4. All tetrads reached stability within 12 days. Social rank was repeatedly analyzed every three to four weeks for a minimum of three consecutive days.

**Social rank in GR<sup>D1Cre</sup> and VTA<sup>inhib</sup> mice.**

Social rank of GR<sup>D1Cre</sup> mice was determined in the tube test from 7 tetrads made of 1 GR<sup>D1Cre</sup> mouse and 3 GR<sup>loxP/loxP</sup> control mice. All the mice were matched by weight and from different litters, thus unknown to each other.

For social rank after dopamine neuron inhibition, tetrads were made of 1 VTA<sup>inhib</sup> mouse and 3 control mice all weight-matched and from the same breeding but not from the same litter (thus unfamiliar). CNO was given either *ip* (1mg kg<sup>-1</sup>) 30 min before tube test to all individuals or was given continuously once tetrads were formed in drinking water (10mg L<sup>-1</sup>) depending on the experiment (see the results section). To estimate the distribution of the number of victories obtained by 7 or 6 tetrads in 27 contests per tetrad (9 per day for three days), we used a substitution method based on stochastic simulations: a surrogate. Simulation is based on a straightforward fighting model where the first mouse won all contests, the second mouse won two out of three contests (against the third and fourth mice), the third mouse won one out of four contests, and the fourth mouse consistently lost all of its contests. We determine the number of victories by randomly selecting one mouse from each tetrad (with an equal distribution between ranks 1, 2, 3, or 4) and conducting 27 contests per tetrad. Subsequently, we calculated the number of wins for seven mice across 7 tetrads. Considering the total number of tetrads (7 tetrads x 9 contests x 3 days), the resulting number of wins ranged from 0 to 378. To obtain the distribution of the number of wins we repeated this estimation process 10,000 times. The null hypothesis assumes that all samples (i.e., number of wins) originate from a uniform distribution of mouse rank. To assess the statistical significance of the observed number of wins in the experimental data, we compared it to the number of wins observed in the surrogate data. The threshold for statistical significance was defined



as being greater than or equal to the number of wins in the surrogate, which means less than 500/100/10 times out of 10,000 simulations (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ).

**Territory urine marking assay.** R1 and R4 mice from a tetrad were placed in an empty PVC box (42x42x15 cm), separated by a central transparent perforated Plexiglas divider, and were let free to explore and mark their territory for 2 hrs. Absorbent paper (Whatman), partially covered with fresh sawdust was set in the bottom of each compartment to collect urine deposited by mice during the session. The absorbent paper was then pictured under UV light (312 nm). The number of urine marks, their total area and their average distances from the midline area were quantified.

**Warm spot occupancy assay.** Tetrads were placed in a transparent plastic cage (35x20x18 cm) without litter and placed on ice (bottom cage temperature 4 °C). 20 minutes later, a warm plate (11x9 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, was scored by an experimenter blind to the conditions.

### **Spontaneous locomotor activity**

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). During three hours, 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.

### **Anxiety-like behavior**

**Dark-Light box.** The dark-light 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 (5x5 cm). Animals were initially placed into the black compartment, and exploration was recorded for 10 min, under 30 lux. The time spent in each compartment was blindly scored by two experimenters.

**Elevated O-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 into 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 minutes and the time spent in 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 the cylinder 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. When tested in animals living in tetrads, the experiment was repeated 24 h later when planned in the experimental design.

### **Sociability, three-chamber 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. On the opposite sides of the 2 lateral compartments, 2 clear perforated plastic boxes (10×7 cm, 7 cm high) were placed. One contained an unfamiliar adult male mouse (C57BL/6J), the other was left empty. During the habituation phase (5 min), the challenged individual was placed in the closed central compartment. Doors were then opened and the mouse was free to explore the display for 5 min. The sessions were recorded, and the close interaction time with the empty box and with the box containing an unknown congener was 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, for 5 minutes.

### **Operant behavior.**

The behavioral procedures were performed in 8 matching operant chambers (model env-307w; Med-Associates, St. Albans, VT) enclosed in a sound- and light-attenuating cabinet equipped with an exhaust fan, which provided 72 dB background white noise inside the chamber. The internal dimensions of the experimental chamber were 22 × 18 × 13 cm, and the floor consisted of metal rods placed 0.87 cm apart. Each chamber was equipped with two retractable levers that could be extended to the left and right of a recessed food magazine. A liquid dipper was used to deliver 20% sucrose solution in the magazine.

**Dipper Training.** Mice, treated with CNO in drinking water (10 mg L<sup>-1</sup>) for two weeks, were first trained to consume the reward from the dipper by placing them inside the chambers with the dipper in the raised position, providing access to a drop of 20% sucrose. The dipper was retracted 10 s after the first head entry into the feeder trough. A variable intertrial interval (ITI) ensued, followed by a new trial identical to the first. The session ended after 30 min or 20 dipper presentations. On the following day, mice received another session similar to the first, except that the dipper was raised for 8 s and then lowered independently of whether or not mice had made a head entry. The session ended after 20 dipper presentations.

**Lever Press Training.** At the beginning of the session, the lever was extended into the chamber, and lever presses were reinforced on a continuous reinforcement schedule (CRF). In this and all subsequent sessions, the reward consisted of raising the dipper for 5 s. The lever was retracted after every two reinforcements and then re-extended after a variable ITI (average 30 seconds). The session ended

when the mouse earned 20 reinforcements or 30 min elapsed. After two CRF sessions, mice then moved to random ratio schedule (RR). Mice performed 2 sessions under a RR5 [ $p(O/R) = 0.2$ ] schedule followed by 2 sessions under a RR10 [ $p(O/R) = 0.1$ ] and 2 sessions under a RR20 [ $p(O/R) = 0.05$ ]. The lever press per minute were recorded during each session.

### **Aggressiveness, resident intruder challenge**

Ranks 1 and 4 males were individually housed for 48 hours before starting the resident intruder test. 10 weeks old 129/SvEv males (Janvier laboratory, Saint-Berthevin, France) were used as intruders. The experimental sessions were carried out between 9.30 am and 3 pm. The intruder was placed in the cage of the challenged mouse, and social interactions were videotaped for 20 minutes for ulterior manual scoring by an experimenter blind to the conditions. A second session was repeated 24 h later with a new intruder. Sessions, in which either R1 or R4 individuals from on tetrad displayed aggressive behavior, were scored.

### **Non-matching to sample T-maze task**

The test was performed as previously described (4). Briefly, mice housed in tetrads underwent for 3 days a moderate food reduction (2 g/mouse/day). A given amount of food pellets (about 2 g per mouse) was daily placed on the floor of the cage so that each individual could easily access it. Mice were daily weighed, taking care not to go below 85% of their initial weight. The 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 (20 mg dustless sugar pellets, Bioserv). For the next three days, mice had to complete 4 forced runs each day, during which one of the two arms was alternatively closed to habituate to the guillotine doors (Figure 3C). The mice were then tested in 10 trials per day. Each trial consisted 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 the 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, the 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**

The social defeat was performed as previously described (5). 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 minutes of 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 seconds 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 was re-exposed to the open field for 150 seconds. The sessions were recorded and the time spent in direct interaction with the boxes was manually quantified by an experimenter blind to the conditions. For hMD4 experiments, animals treated with CNO in the drinking water (10 mg L<sup>-1</sup>) but that have not been exposed to repeated social defeat were used as controls.

### **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 as previously described (see “Spontaneous locomotor activity above). Animals were habituated to the apparatus for 3 hours on 2 consecutive days and received a saline injection on day 2 (NaCl 0.9% saline solution, 10 ml kg<sup>-1</sup>, *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<sup>-1</sup> *i.p.*) and left inside during 180 minutes after injection. Following 7 days of withdrawal, mice received a challenging injection of cocaine (10mg kg<sup>-1</sup> *i.p.*). On the following day, mice received a second challenging injection of cocaine with 10mg kg<sup>-1</sup> for rank 1 and 12,5 10mg kg<sup>-1</sup> for rank 4 individuals. The behavioral sensitization experiment has been carried out from 9 am to 1 pm.

### ***In vivo* electrophysiological recordings**

Three to 5 months old R1 and R4 mice were anesthetized with chloral hydrate (8%), 400 mg kg<sup>-1</sup> *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 (3)). 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 microdrive. 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 the Spike2 program. (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) (6). 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>). DA 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 the 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 msec whenever and they terminate with an inter-spike interval >160 ms. The firing rate and %SWB of each recorded neuron are used as independent variables. Normality of the data set is tested by Shapiro test. For firing frequency, comparison between group is made using Student's t-test (for the normal data set) or Wilcoxon signed-rank test (for non-normal distribution). For %SWB, because the data set exhibit skewed distribution, comparison between group is made using the surrogate method. We first calculate the absolute difference of mean %SWB between the two groups ( $\Delta\langle\%SWB\rangle_o$ ). We then generated 10 000 surrogate data ( $\Delta\langle\%SWB\rangle_s$ ). A surrogate data is obtained by resampling two groups from the original dataset (by permutation) and for each resampling by calculating the difference in the mean %SWB of the two resampled group. Original dataset is obtained by pooling the %SWB observed in Rank 1 and Rank 4 mice (or in VTA<sup>inhib</sup> and control) and resampling by randomly re-assign these values to two groups. We then calculate 10 000 surrogate values  $\Delta\langle\%SWB\rangle_s$  and count the number of times  $\Delta\langle\%SWB\rangle_s \geq \Delta\langle\%SWB\rangle_o$ . The null hypothesis is that all samples (From Rank 1 and Rank 2) come from the same distribution. The difference reaches statistical significance if the surrogate reproduces the absolute difference of mean %SWB less than 500/100/10 times over 10 000 simulations (\*p<0.05, \*\*p<0.01, \*\*\*p<0.001).

### **Ex vivo patch-clamp recordings.**

Six weeks old DATiCre mice were injected bilaterally into the VTA with AVV-DIO-hM4D-mCherry. Four weeks after mice were anesthetized (Ketamine 150 mg/kg/ Xylazine 10 mg/kg) and transcardially perfused with aCSF for slice preparation. For VTA recordings, horizontal 250  $\mu\text{m}$  slices were obtained in bubbled ice-cold 95% O<sub>2</sub>/5% CO<sub>2</sub> aCSF containing: KCl 2.5 mM, NaH<sub>2</sub>PO<sub>4</sub> 1.25 mM, MgSO<sub>4</sub> 10 mM, CaCl<sub>2</sub> 0.5 mM, glucose 11 mM, sucrose 234 mM, NaHCO<sub>3</sub> 26 mM. Slices were then incubated in aCSF containing: NaCl 119 mM, KCl 2.5 mM, NaH<sub>2</sub>PO<sub>4</sub> 1.25 mM, MgSO<sub>4</sub> 1.3 mM, CaCl<sub>2</sub> 2.5 mM, NaHCO<sub>3</sub> 26 mM, glucose 11 mM, at 37 °C for 1 h, and then kept at room temperature. Slices were transferred and kept at 32–34 °C in a recording chamber, superfused with 2.5 ml min<sup>-1</sup> aCSF. Visualized whole-cell current-clamp recording techniques were used to measure excitability using an upright microscope (Olympus France). DA neurons were recorded in the lateral VTA and identified for the expression of mCherry (AAV-hSyn-DIO-hM4D-mCherry). We compared the excitability of each VTA DA neurons induced by consecutive current injections before and after the bath application of CNO (30  $\mu\text{M}$ ) during 10 min.

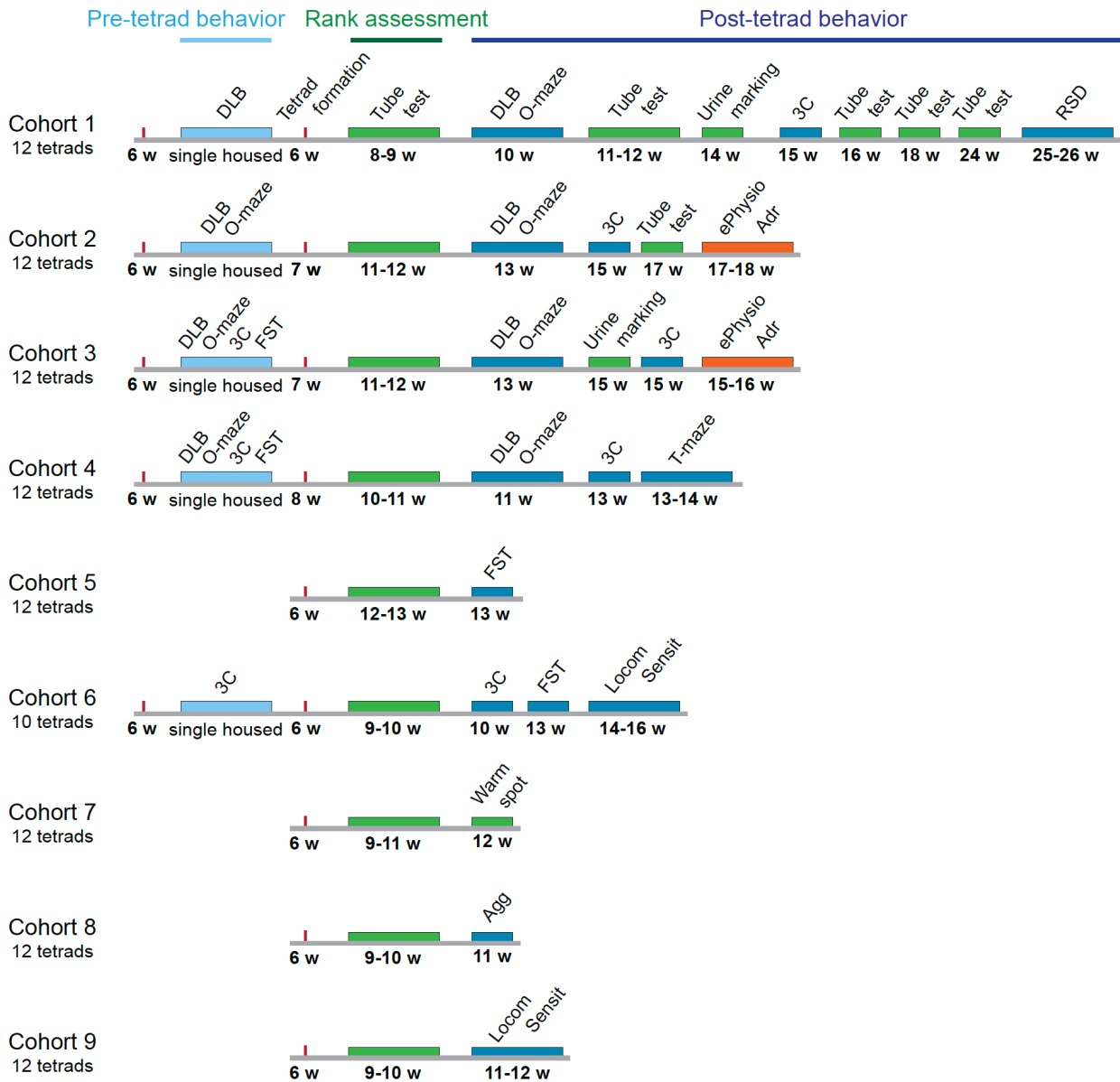
### **Immunostaining**

Following CNO treatment, anesthetized mice were perfused (intra-cardiac) with 4% paraformaldehyde. Brains were removed, post-fixed overnight in the same solution, and sliced with a vibratome (30  $\mu\text{m}$ ). Sections containing SN and VTA were incubated with antibodies directed against tyrosine hydroxylase (mouse monoclonal, 1:1000, Merck-Millipore MAB318) and mCherry (rabbit polyclonal, 1:500, Abcam

ab167453) overnight at 4°C with constant shaking. Sections were then incubated for 2 h at room temperature with anti-mouse Alexa 488 (goat polyclonal, Invitrogen A11001, 1:500) and anti-rabbit Cy3 (Invitrogen A10520, 1:500) antibodies. Sections were mounted using Vectashield with DAPI (Vectorlaboratories) and analyzed by fluorescence microscopy.

### **Statistical Analysis**

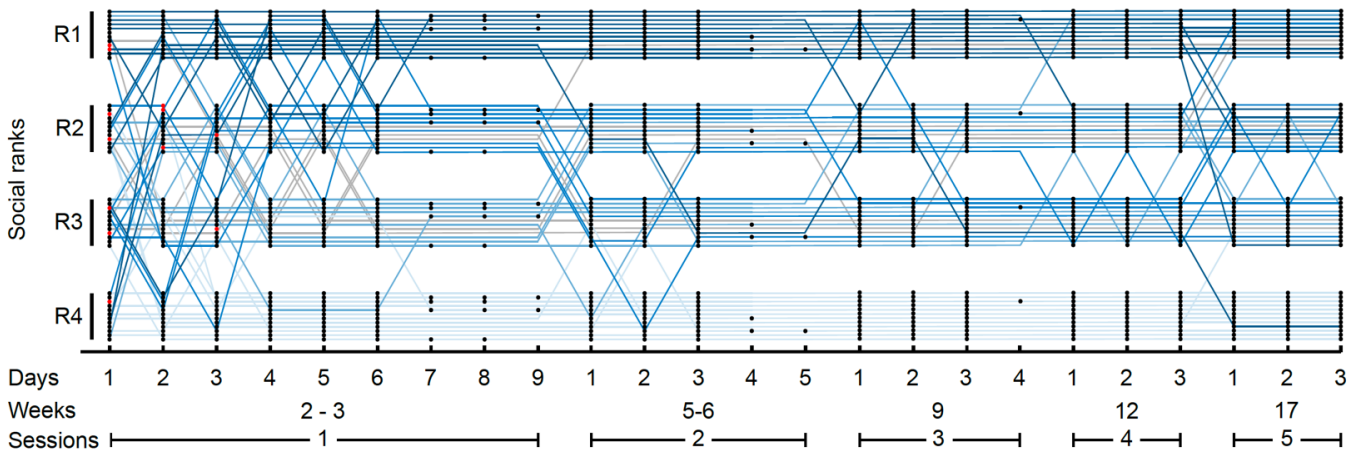
Data are expressed as mean  $\pm$  SEM. Statistical analyses were performed by using the Mann-Whitney non-parametric test, the Gehan-Breslow-Wilcoxon test, the Wilcoxon matched-pairs signed rank test, the Kruskal-Wallis test, or one or two-way ANOVAs. In this case, when the primary effect was found to be significant, post hoc comparisons were made using a Bonferroni/Dunn test. Differences of  $p \leq 0.05$  were considered statistically significant. Statistical analyses were carried out using PRISM software except when indicated (with R). For each panel, statistical parameters are detailed in the data set, except for surrogate data testing that are detailed above in the corresponding Material and Method section.



**Figure. S1 Timeline of the experiments performed with ranked tetrads of C57B/L6J mice.**

For each cohort, green boxes indicate ranking experiments (tube-tests, urine marking and warm-spot occupancy assay), blue boxes indicate behavior experiments (DLB : Dark-light box test, O-Maze : Elevated O-Maze; 3C: Three-chambers test, RSD : Repeated social defeats, FST : Forced-swim test, T-Maze : Non-matching to sample T-maze task, Locom sensit : Locomotor sensitization to cocaine, Agg : aggressive behavior tests), and orange boxes indicate *in vivo* electrophysiology measurements (ePhysio) and adrenal glands weighting (Adr). Below, the ages in weeks (w) are indicated.

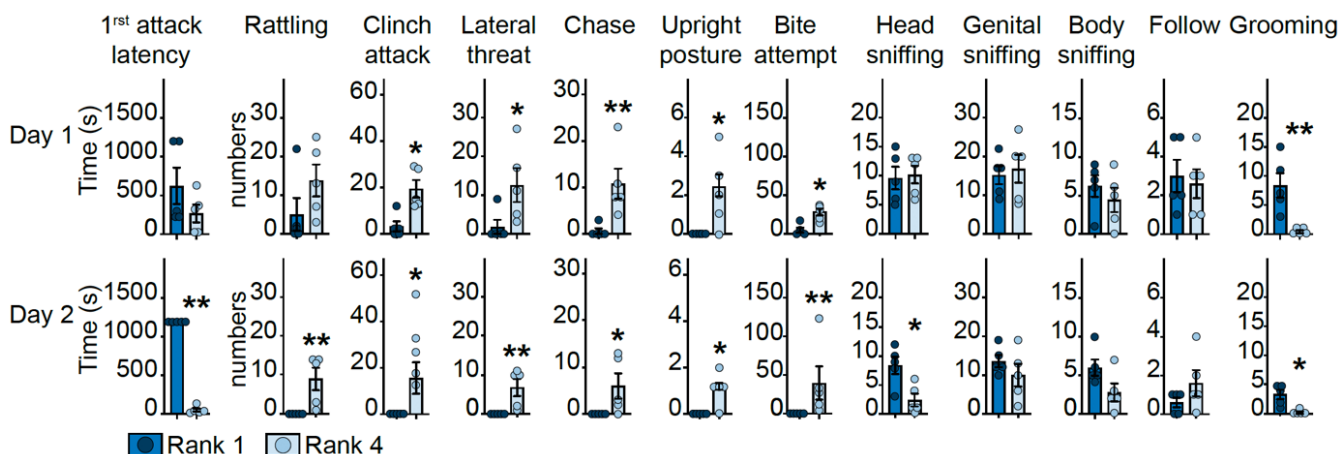
### Social ranks over time



**Figure S2. Stability of hierarchical classification and territoriality**

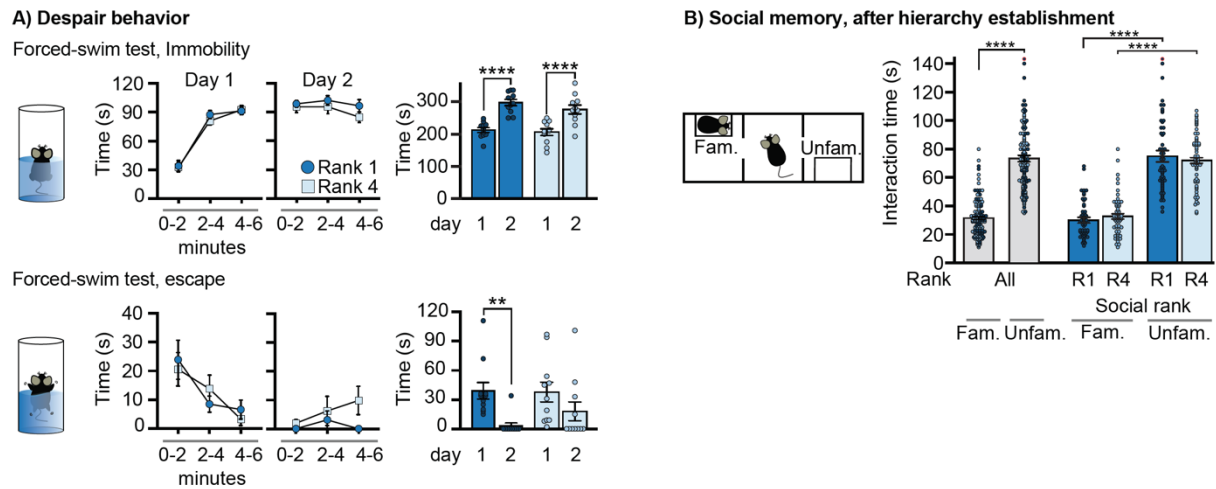
Rank assessment in tube-test is shown with a daily resolution for 12 tetrads. As in Figure 1, each line corresponds to an individual mouse and indicates the tetrad to which it belongs (1 to 12). The five sessions of tube-tests measurement and their length in days are indicated below. The blue intensity indicates its rank attainment after the first session. R1-4 indicates its social rank. Black dots on the lines indicate that a tube-test was performed during the corresponding day. Note that there is no dot after the hierarchy has reached the stability criterion during a session (*i.e.* that R1 and R4 mice were stable for three consecutive days, with an initial period of at least 6 days of testing). Grey lines correspond to mice of intermediate ranks that did not reach stability at the end of the first session. On days 1, 2, and 3, red dots indicate individuals for whom the rank could not be determined.





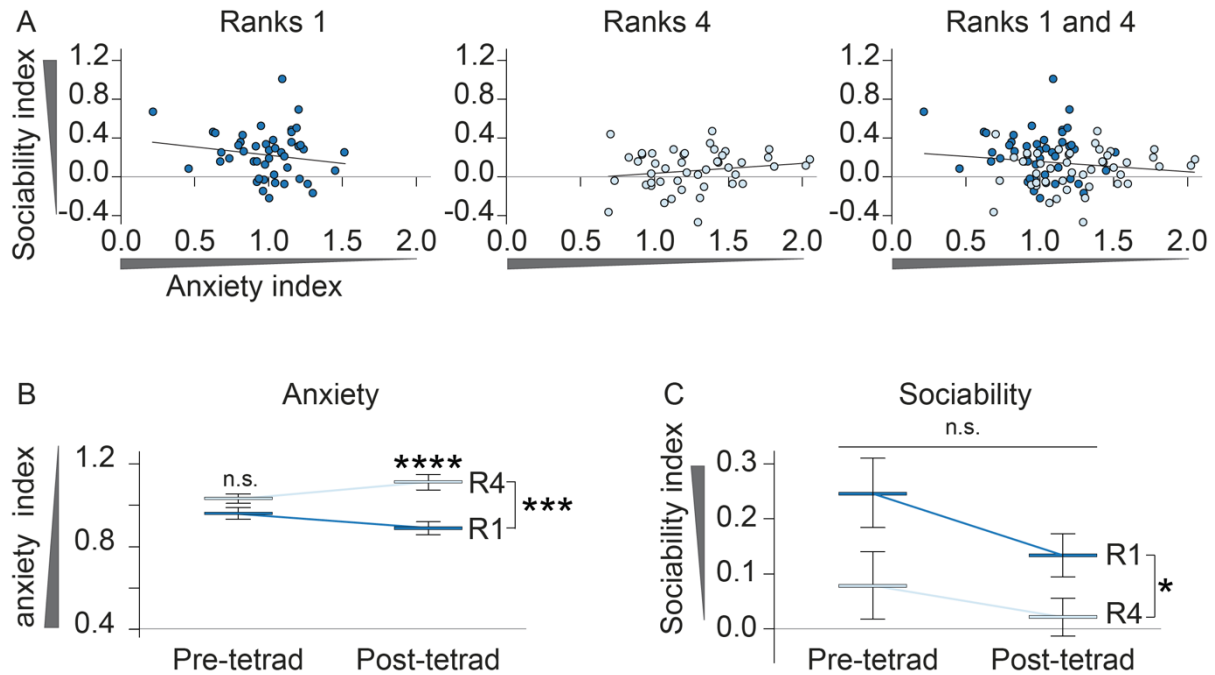
**Figure S3. The aggressiveness of rank 1 and rank 4 mice.**

The latencies to the first attack during resident-intruder challenges were scored for rank 1 (dark blue) and rank 4 individuals (light blue, left graph). The occurrences of aggressive (rattling, clinch attack, lateral threat, chase, upright posture, bite attempt) and pre-social behaviors (sniffing, follow, grooming) were scored during 20 min. Data are expressed as mean ± SEM. Statistical analyses were performed by using the Mann-Whitney non-parametric test. \*p < 0.05; \*\*p < 0.01 (n = 5 per group).



**Figure S4. Social memory and despair behavior of rank 1 and rank 4 mice.**

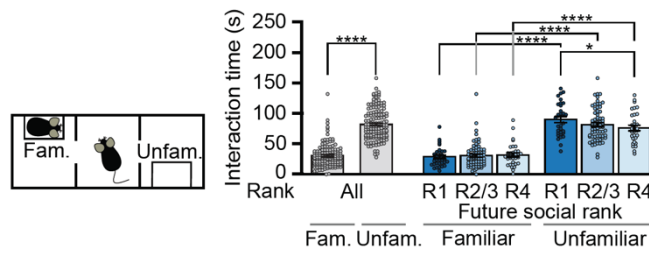
(A) Highest and lowest ranked individuals display similar depression-like behavior in the forced-swim test. After rank establishment (left), the time of immobility in water is presented in 2 minutes bins (upper panel, left) and for the 6 minutes of the test (upper panel, right) for R1 and R4 mice ( $n=11$  per group). Results obtained on the first day (Day 1) and 24 h later (Day 2) are shown. Time course-day 1: main time effect  $F(2, 40)=107.2$ ,  $p<0.0001$ . Time course-day 2: No effect. Two-way repeated ANOVA. Total time: main day effect  $F(1, 20)=97.41$ ,  $p<0.0001$ . Two-way repeated ANOVA followed by Bonferroni correction day1 vs day 2 \*\*\*\* $p<0.0001$ . The lower panels show the quantification of escape behavior of the same individuals. Time course-day 1: main time effect  $F(2, 40)=0.207$ ,  $p<0.001$ . Time course-day 2: No effect. Two-way repeated ANOVA. Total time: main day effect  $F(1, 20)=13.14$ ,  $p<0.01$ . Two-way repeated ANOVA followed by Bonferroni correction day1 vs day 2 \*\* $p<0.0001$ . (B) Highest and lowest ranked mice display similar social memory. The duration of time spent interacting with a familiar (Fam.) and an unfamiliar mouse (Unfam.). C57B/L6 (grey,  $n=115$ ), R1 (dark blue,  $n=57$ ) and R4 (light blue,  $n=58$ ) individuals. All mice: Wilcoxon matched-pairs signed rank test, \*\*\*\* $p<0.0001$ . Right graph: Main familiarity effect  $F(1, 113)=231.1$ ,  $p<0.000$ . Two-way repeated ANOVA followed by Bonferroni correction, R1 and R4 fam. vs unfam. \*\*\*\* $p<0.0001$ . Error bars,  $\pm$  SEM.



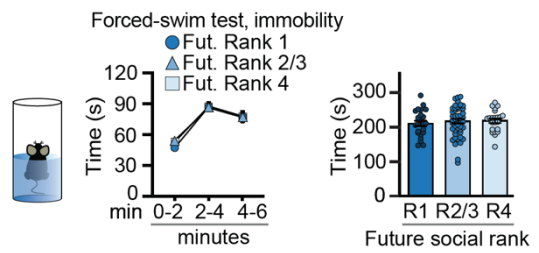
**Figure S5 Correlation between sociability and anxiety and evolution pre- vs post-tetrad**

**(A)** Correlations between sociability and anxiety in R1, R4 and pooled R1 and R4 mice. Sociability index is calculated as  $(IT_{\text{mouse}} - IT_{\text{empty box}})/(IT_{\text{mouse}} + IT_{\text{empty box}})$  where IT is the interaction time. The anxiety index is the average of the normalized scores on dark-light box and elevated O maze tests. Left panel:  $R=-0.17$ , Pearson correlation test  $p=0.26$ . Middle panel:  $R=0.17$ ,  $p=0.33$ . Right panel:  $R=-0.14$ ,  $p=0.13$  **(B)** Analysis of sociability, pre- and post-tetrad formation.  $n=34$  mice for R1 and R4. Main social rank effect  $F(1, 66)=6.63$ ,  $*p<0.05$ . Two-way repeated ANOVA. Error bars,  $\pm$  SEM. **(C)** Analysis of anxiety pre- and post-tetrad.  $n=48$  mice for R1 and R4. Anxiety indexes were scored as described above except that the values for anxiety were not normalized upon average R1 scores for each cohort but normalized with the average scores for all individuals in pre-tetrad for the pre-tetrad scores and in post-tetrad for the post tetrad scores. Main social rank effect  $F(1, 94)=15.28$ ,  $***p<0.001$ ; no effect of pre-post tetrad  $F(1, 94)=0.019$ ,  $p=0.89$ ; interaction effect  $F(1, 94)=7.78$ ,  $p<0.01$ . Two-way repeated ANOVA followed by Bonferroni correction R1 vs R4 in post-tetrad  $****p<0.0001$ . Error bars,  $\pm$  SEM.

**A) Social memory, before hierarchy establishment**

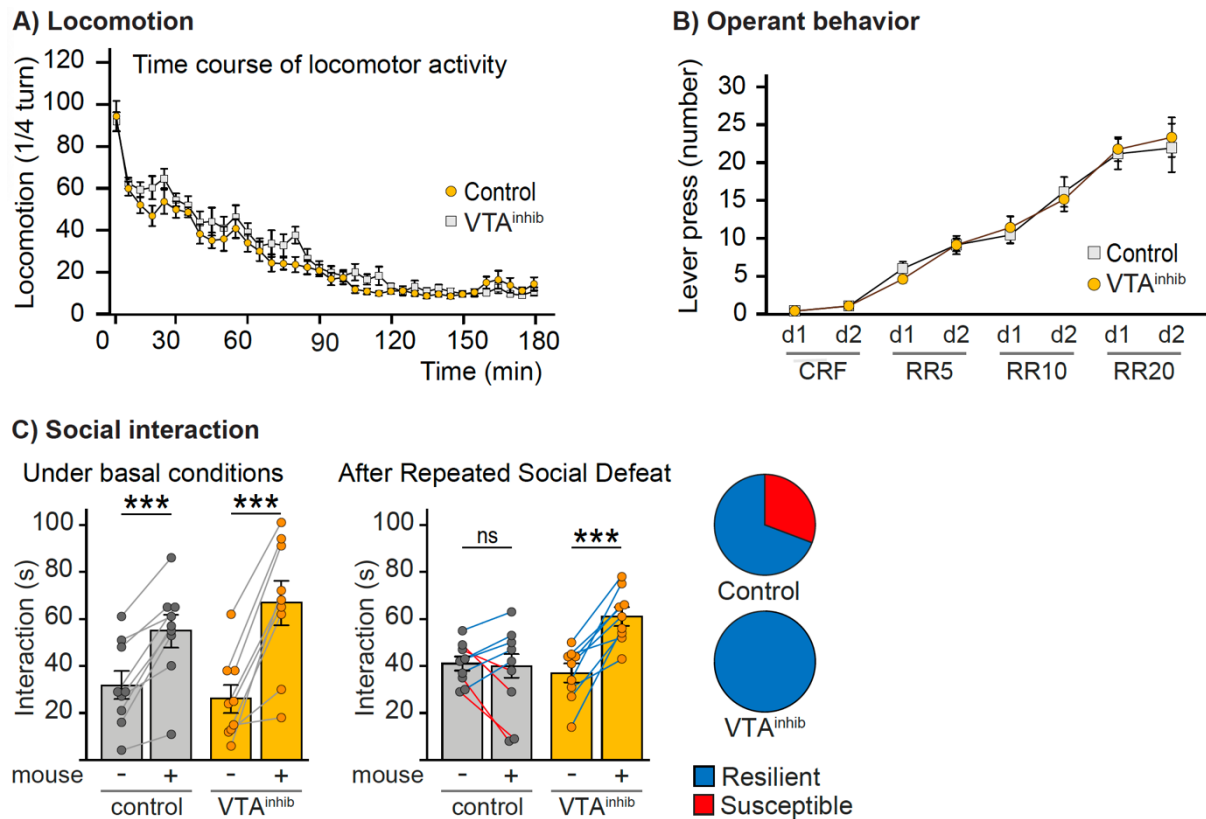


**B) Despair behavior, before hierarchy establishment**



**Figure S6. Social memory and despair behavior of future highest and lowest-ranked individuals.**

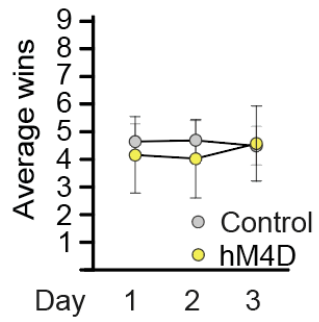
**(A)** Future rank 4 and future R1 individuals display similar social memory before rank establishment. The time length interaction with the box containing a familiar (Fam.) social cue vs a box containing an unfamiliar (Unfam.) mouse is shown. All mice: Wilcoxon matched-pairs signed rank test, \*\*\*\* $p < 0.0001$ . Right graph: main familiarity effect  $F(1, 133) = 291.6$ ,  $p < 0.0001$ . Two-way repeated ANOVA followed by Bonferroni correction, R1, R2/3 and R4 fam. vs unfam. \*\*\*\* $p < 0.0001$ ; R1 unfam. vs. R4 unfam. \* $p < 0.05$ . Error bars,  $\pm$  SEM. **(B)** Before rank establishment (right), future rank 1 and rank 4 individuals display similar despair behavior. The time of immobility is shown for each bin of two minutes (lines), and for the 6 minutes of the test (bars). Future R1 ( $n = 24$ ), Future R4 ( $n = 24$ ), and Future R2/3 ( $n = 48$ ) (dark, medium and light blue, respectively). Time course: main time effect  $F(2, 186) = 69.53$ ,  $p < 0.0001$ . Two-way repeated ANOVA. Total time: Kruskal-Wallis non-parametric test,  $p = 0.56$ . Error bars,  $\pm$  SEM.



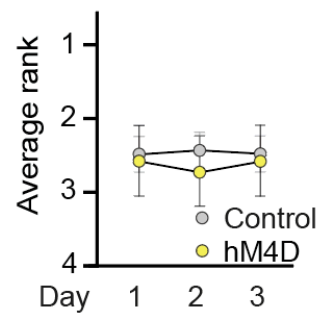
**Figure S7. Behavioral consequences of chemogenetic inhibition of VTA dopamine neurons.**

**(A)** Inhibition of dopamine neurons does not induce locomotor activity changes. No effect of group  $F(1, 16)=2.785, p=0.11$ ; no interaction time  $\times$  group  $F(35, 560)=1.059, p=0.38$ . Two-way mixed ANOVA,  $n=9$  mice per group. Error bars,  $\pm$  SEM. **(B)** Mice with dopamine neurons inhibition show normal level of lever press for food reward. Main effect of reinforcement schedule  $F(7, 112)=103.2, p<0.0001$ ; no effect of group  $F(1, 16)=0.003, p=0.96$ ; no interaction time  $\times$  group  $F(7, 112)=0.312, p=0.95$ . Two-way mixed ANOVA,  $n=9$  mice per group. Error bars,  $\pm$  SEM. **(C)** Left graph: Inhibition of dopamine neurons did not induce changes in social interaction under unstress conditions. Interaction time with an empty box (mouse -) or a CD1 mouse (mouse +). Main effect of social cue  $F(1, 16)=73.31, p<0.001$ ; no effect of group  $F(1, 16)=0.105, p=0.75$ . Two-way repeated ANOVA followed by Bonferroni correction,  $n=9$  mice per group,  $***p<0.001$ . Right graph: repeated social defeat decreases social interaction in control mice but not in mice with inhibited dopamine neurons activity. Main effect of social cue  $F(1, 16)=16.38, p<0.001$ ; no group effect  $F(1, 16)=3.171, p=0.09$ ; interaction  $F(1, 16)=18.94, p<0.001$ . Two-way repeated ANOVA followed by Bonferroni correction,  $***p<0.001$ . Error bars,  $\pm$  SEM. Pie charts: % of resilient (in blue) and susceptible mice (in red) after repeated social defeats in controls and in mice with inhibited dopamine neurons.

A) Stabilization of wins within hM4D tetrads



B) Stabilization of ranks within hM4D tetrads



**Figure S8. Stabilization of wins within hM4D tetrads.**

**(A)** Average wins and **(B)** Average rank upon hierarchy stabilization before CNO treatment. Control and hM4D mice: n=21, and n=7, respectively. Error bars,  $\pm$  SEM.

## SI References

1. Turiault M, *et al.*, Analysis of dopamine transporter gene expression pattern – generation of DAT-iCre transgenic mice: Cre-mediated recombination in dopaminergic cells. *FEBS J.* 274, 3568–3577 (2007).
2. Lemberger T, *et al.*, Expression of Cre recombinase in dopaminoceptive neurons. *BMC Neurosci.* 8, 4 (2007).
3. Watson C, Paxinos G, *Chemoarchitectonic atlas of the mouse brain.* (Elsevier Academic Press, 2010).
4. Sigurdsson T, Stark KL, Karayiorgou M, Gogos JA, Gordon JA, Impaired hippocampal–prefrontal synchrony in a genetic mouse model of schizophrenia. *Nature* 464, 763–767 (2010).
5. Barik J, *et al.*, Chronic stress triggers social aversion via glucocorticoid receptor in dopaminoceptive neurons. *Science* 339, 332–335 (2013).
6. -Grace A, Bunney B, The control of firing pattern in nigral dopamine neurons: burst firing. *J. Neurosci.* 4, 2877–2890 (1984).