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## ► 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*, 2023, 10.1016/j.biopsych.2023.08.029 . hal-04284656v1

**HAL Id: hal-04284656**

**<https://hal.science/hal-04284656v1>**

Submitted on 16 Nov 2023 (v1), last revised 28 Nov 2023 (v2)

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

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## **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 mouse translates into individual and interdependent behavioural 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 behaviours 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 behavioural 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 behavioural response to cocaine and a decreased vulnerability to depressive-like behaviours following repeated social defeats. The pharmacogenetic inhibition of this neuronal population and the genetic inactivation of glucocorticoid receptor signalling 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 defence and exhibit a large repertoire of social behaviours, including physical exploration, vocal communication, aggression, imitation, etc. The social rank of individuals can be determined based on precedence behaviours, 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 on the individual behavioural fate (10)(11). In congenic mice, specific behavioural patterns emerge in mice raised in large groups (12)(13), and hierarchy-based differences in behavioural 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 behavioural diversity in 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 to the emergence of social hierarchy and behavioural diversity and may constitute a link between social status and mental health. This brain system modulates a broad spectrum of behaviours, 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 for 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 toward a role for brain regions belonging to the meso-corticolimbic reward system in social hierarchy, the contribution of VTA dopamine neurons themselves has never been investigated.

In this study, we examine the segregation of individual behaviours with social status in colonies of four genetically identical male mice (tetrads). We investigated whether pre-existing behavioural 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 signalling in the establishment of social hierarchy and individuation of behaviours.

## Methods and Materials

Methods are detailed in supplementary information.

## Results

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

We analysed social ranks and behaviours in tetrad colonies of four weight 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 analysed the social ranks of animals using a precedence test based on encounters within a plastic tube between each possible congener pairs 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 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 with 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 behaviour 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 behaviours 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. From 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 behaviours, including clinch attacks, lateral threats, chases, *etc.* whereas R1 individuals had more prosocial behaviours (*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 behavioural differences.**

Behaviours tested in laboratory settings on isogenic mice, including anxiety-like, sociability, or locomotion display high interindividual variability, 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 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 behaviours pre-exist to social rank establishment**

The behavioural 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 behaviours 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 behaviour between future R1 and R4 individuals (Figure S6B).

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

In human, low social status, is associated with reduced life span (24) and negative health consequences including psychopathologies. In a variety of 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 mg.kg<sup>-1</sup>) 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 neurons 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 signalling 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 neurons activity and higher social rank, we assessed the impact of inhibiting this cell population on social status. We Gi-coupled hM4D receptor, specifically in VTA dopamine neurons, by injecting a Cre-dependant adeno-associated viral vector (AAV8-hSyn-DIO-hM4D(Gi)-mCherry) into the VTA of DATiCre mice (31) (VTA<sup>inhib</sup> mice, Figure 5A, left). 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 neurons 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 neurons 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,  $VTA^{inhib}$  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  $VTA^{inhib}$  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  $VTA^{inhib}$  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 for the lowest and highest ranked individuals, but frequent for the intermediate ranks. Behavioural 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 behaviours 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 behaviours and has well-known anxiolytic properties (38), and optogenetic 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 behaviour and is anxiogenic (41).

A central but poorly explored question is whether the emergence of social ranks precedes the appearance of specific individual behavioural 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 behaviour (43).

Although the literature is less abundant than for males, female colonies form social hierarchies that can be identified with tube tests, may be more robust than in males (33) and do affect their behavioural responses to stress (63). 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 dopaminoceptive neurons in this region play distinct roles depending on whether they express dopamine receptor 1 or 2, determining dominance and subordination, respectively (44). 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 (45). In ants, brain dopamine concentration is higher in socially dominant individuals (46)(47). In striatal structure of birds and lizards, higher ranked individuals have increased levels of dopamine (48)(49). In line with our results, dominant rats have reduced levels of dopamine in the NAcc of (50).

Genetic evidence also sustains a link between dopaminergic neurotransmission and social status. Dopamine transporter gene inactivation in mouse disorganizes social colonies (51), and genetic variants in macaques are associated with social dominance (52). 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 (53)(54). Neuropharmacological approaches also suggest a role for dopamine signalling 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 (55) whereas injection into the NAcc of an agonist did not have an effect in rats (56). Similar experiments with a D1-receptor antagonist facilitated or did not modify social dominance in mice and monkeys (57) whereas injection into the NAcc of an agonist increased dominance in rats (56). Interestingly, changes in VTA dopamine cells activity are observed during the emergence of behavioural 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 behavioural psychopathologies, including addiction and depression that develop with a high interindividual variation that is not fully understood (26)(58). The lower cocaine sensitization of R1 individuals is coherent with their reduced dopamine neuron activity (59). For repeated social defeat, optogenetic stimulation of VTA neurons projecting to the NAcc induces a susceptible phenotype (19) whereas optogenetic (19) or chemogenetic 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)(64). In human, higher socio-economic status has been linked to lower evening glucocorticoid levels (65). Studies in military leaders, as well as in influential individuals from a Bolivian forager-farmer population showed lower glucocorticoid levels (66)(67). These findings are in line with the fact that mice deprived of GR signalling in dopamine-innervated regions exhibit higher social ranking when raised in tetrads or in dyads (68). 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 dopamine pathway might play a principle organizational role in shaping the behavioural trajectories leading to the establishment of social ranking. Altogether, our results points dopamine as a key player in determining social fate of individuals, a process that may occur under the continuous influence of the glucocorticoid stress-response.

## Acknowledgments

The authors warmly thank S. Bhattacharya, C Sandi, N Faucon, R Meloni, L. Amar and S. Vyas for helpful discussions and S.B., L. A., N. F. and R. M. and S.V. for critical reading of the manuscript. Authors wish to thank F. 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 to FT, ANR-14-CE35-0029-01 to SP and ANR-15-CE16-0017 to FT). TC is a recipient fourth-year Ph.D. fellowship from the FRM (FDT202106012968).

### **Author contributions**

DB, CV, CN, AZ, ACM, SM, AF, GD, FM, TC, JPT, **TR, DL, 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.

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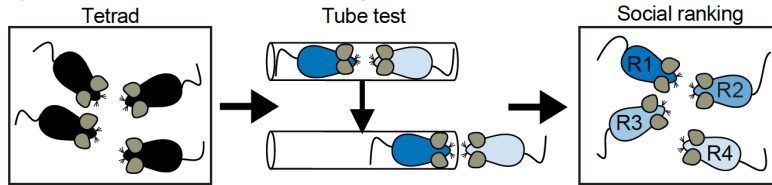
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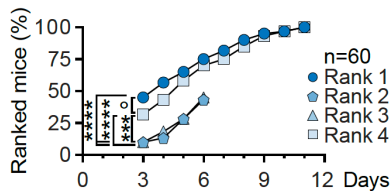
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## Figures

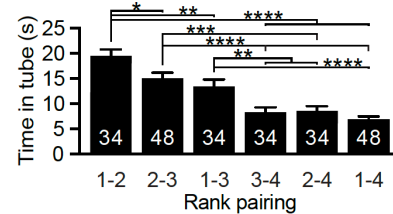
### A) Establishment of social hierarchy in mouse



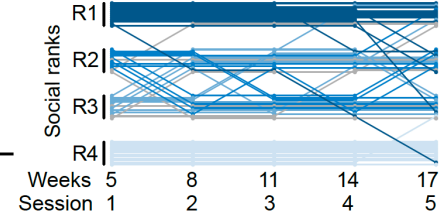
### B) Stabilisation of ranking in tube test



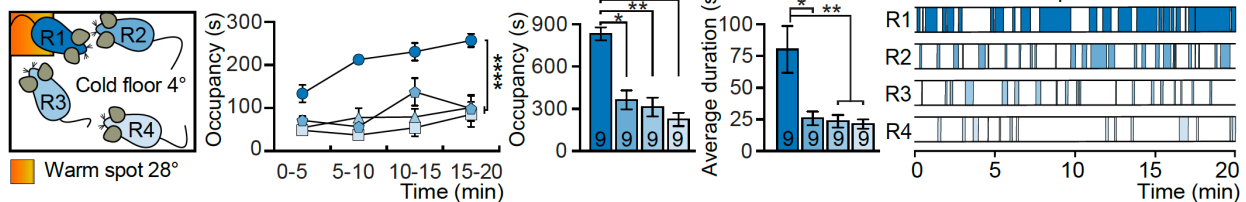
### C) Duration of encounters



### D) Social rank over time



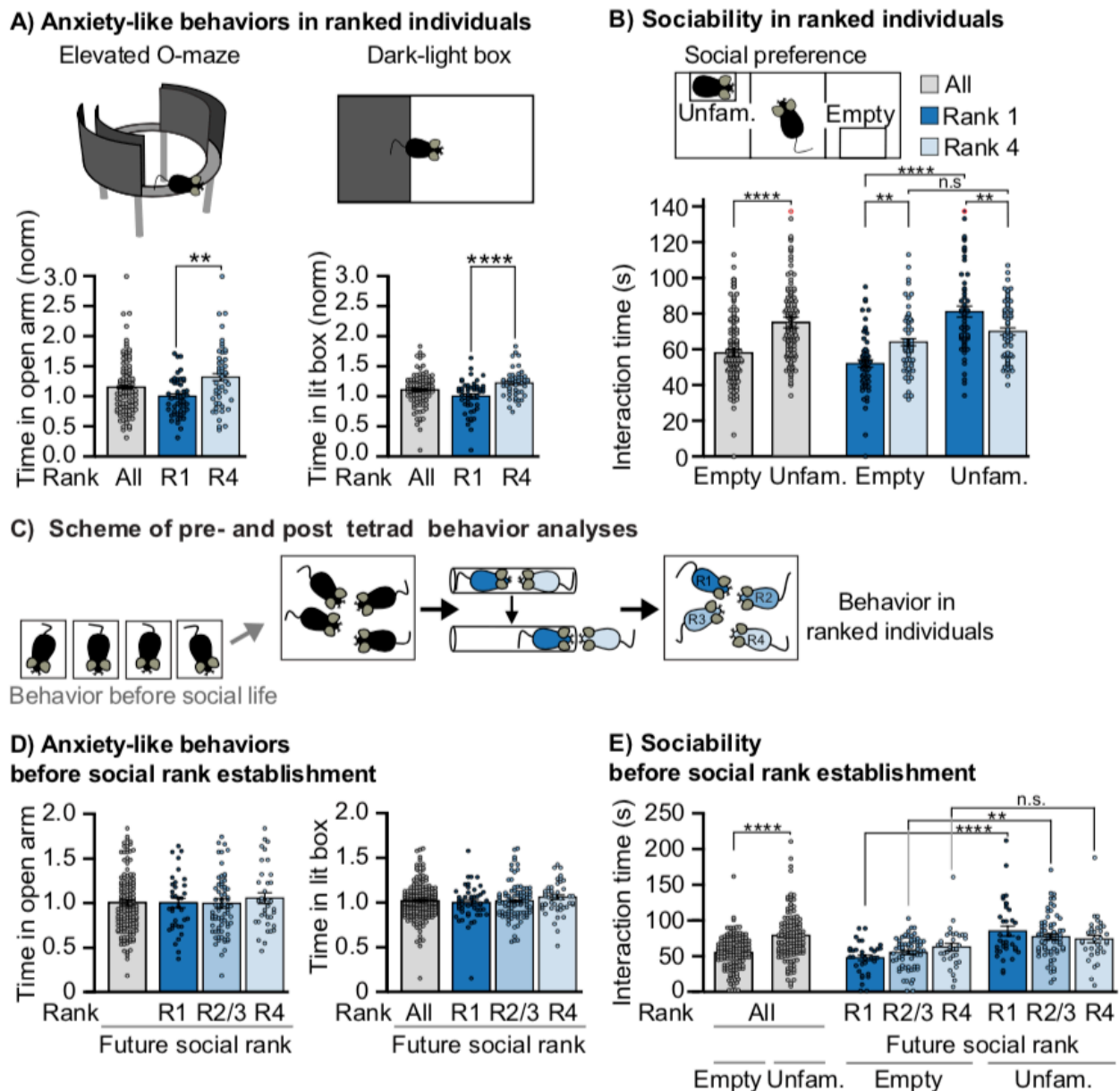
### E) Warm spot occupancy



**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  $^{\circ}p=0.08$ ; 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 analysed 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.



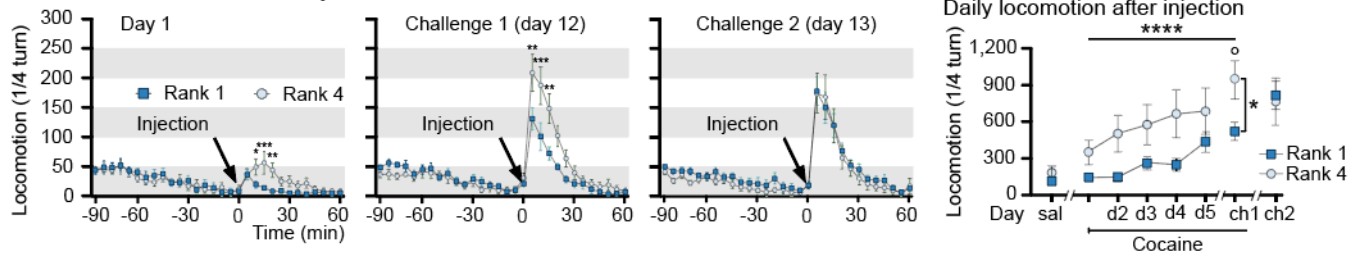
**Figure 2. Differences in sociability between highest and lowest-ranked individuals preexist to rank establishment whereas anxiety emerges from social life within tetrads.**

**(A)** Rank 1 individuals display increased anxiety-like behaviours. 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-chambers test is depicted. The duration of time spent interacting with an empty box (Empty) and with a box containing an unfamiliar mouse (Unfam.) are 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

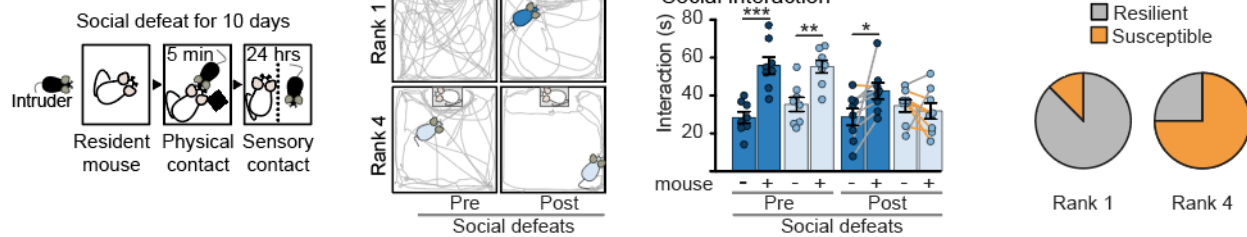
ANOVA followed by Bonferroni correction, R1 empty vs unfam \*\*\*\* $p < 0.0001$ ; R1 vs R4 \*\* $p < 0.01$ . Error bars,  $\pm$  SEM. **(C)** Before being grouped in tetrads, behaviour was assessed in singly housed individuals. **(D)** Before rank establishment, future R1 and future R4 individuals display similar anxiety-like behaviours. 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  $\times$  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

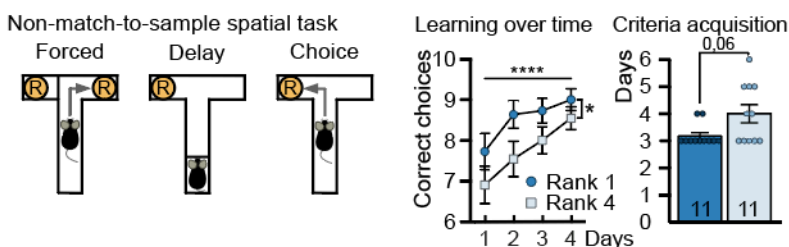
Time course of locomotor activity



### B) Repeated social defeat



### C) Spatial working memory



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

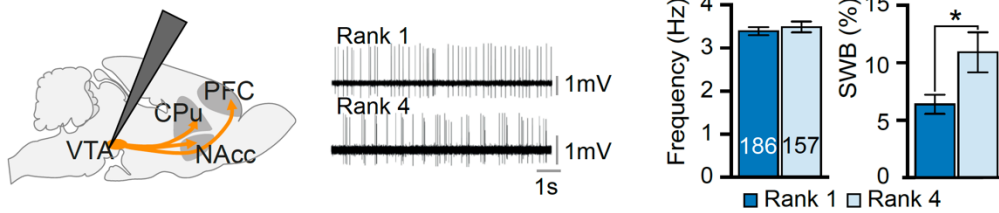
**(A)** Locomotor sensitization to cocaine. Left, time course of Rank 1 ( $n=10$ ) and Rank 4 ( $n=9$ ) individuals locomotion expressed as  $\frac{1}{4}$  turn within a circular corridor for indicated sessions. Time 0 corresponds to the injection of cocaine ( $10 \text{ mg kg}^{-1}$ ), except for Challenge 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 behaviour 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

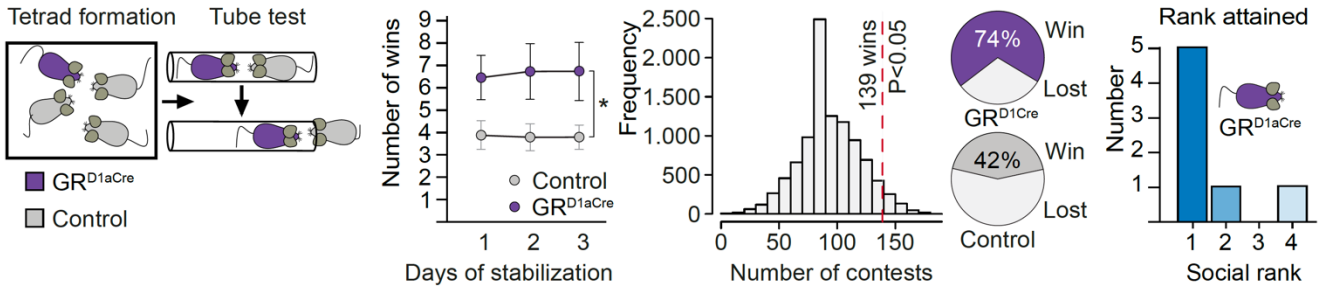


$F(1, 14)=0.76, p=0.40$ . No interaction  $F(1, 14)=1.04, p=0.33$ . Two-way repeated ANOVA followed by Bonferroni correction, empty box vs social cue for R1 mice \*\*\*  $p<0.001$ ; empty box vs social cue for R4 mice, \*\* $p<0.01$ . Post-social defeats: no effect of social cue  $F(1, 14)=1.89, p=0.19$ . 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.

**A) Rank 1 mice have lower VTA dopamine neurons bursting activity**



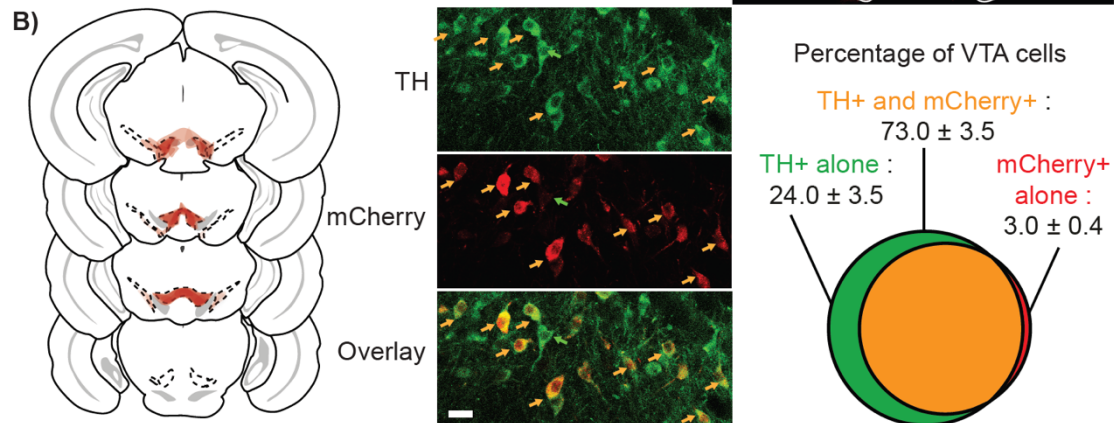
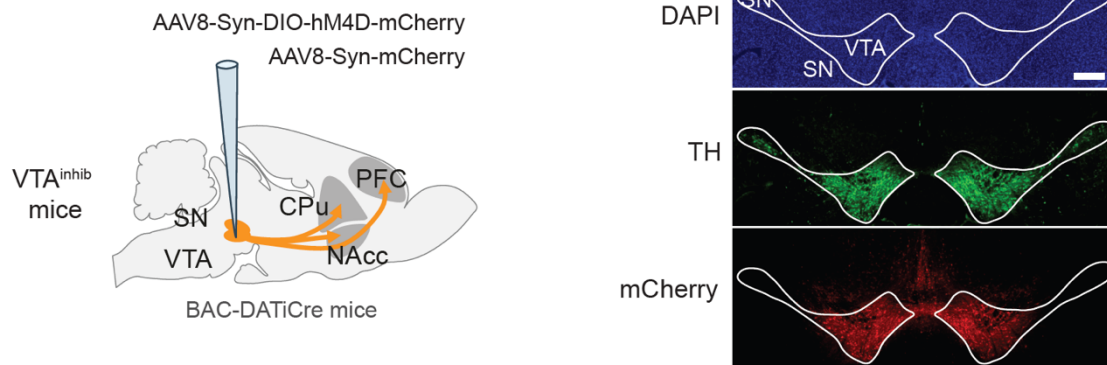
**B) GR deletion in dopaminergic neurons upwards social ranking**



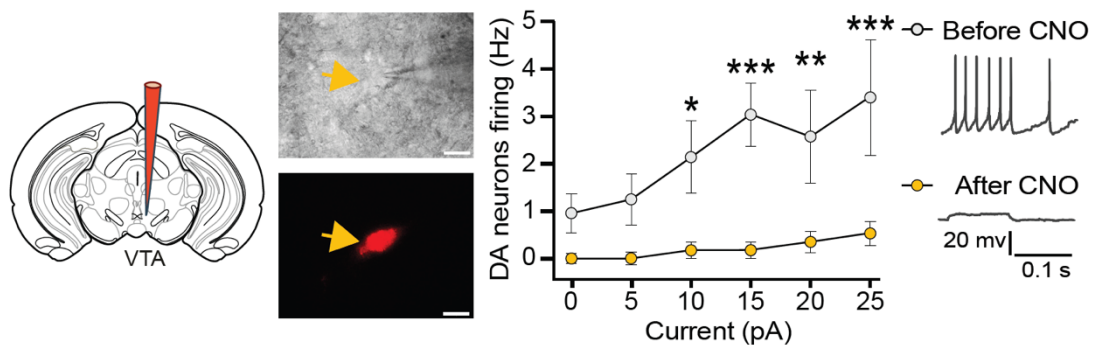
**Figure 4. Dopamine neuron activity in the ventral tegmental area and gene Glucocorticoid Receptor in dopaminergic neurons modulate social rank attainment.**

**(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: \* $p < 0.016$  ( $\Delta < \%SWB >_0 \geq 4.5\%$ , 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>lox/lox</sup>) mice, for three weeks before tube test. Number of tetrads = 7 (GR<sup>lox/lox</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.

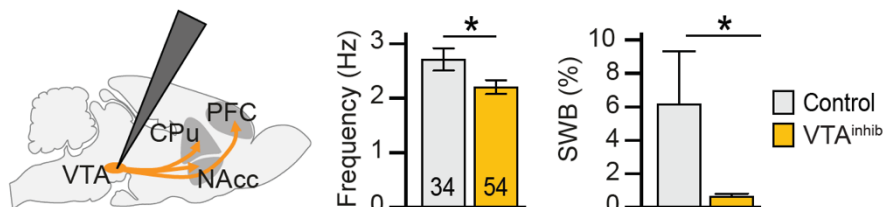
**A) DREADD approach in VTA dopamine neurons**



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



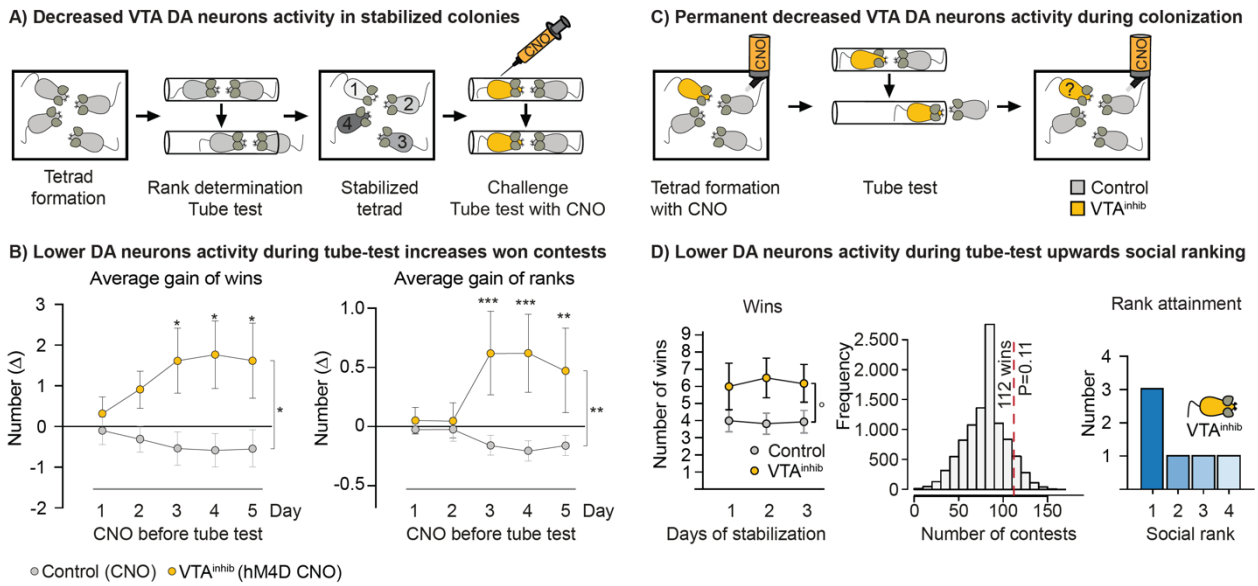
**D) In vivo DREADD mediated VTA dopamine neurons inhibition by CNO**



**Figure 5. DREADD mediated VTA dopamine neurons 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). **(B)** *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. **(C)** *In vivo*, selective chemogenetic inhibition prevents dopamine neurons firing in brain slices. CNO treatment (10 mg L<sup>-1</sup> during 10 to 15 days) in drinking water significantly reduced spontaneous firing frequency, and almost abolished bursting activity of VTA dopamine neurons in anesthetized VTA<sup>inhib</sup> mice when compared to control mice (Figure 5D). VTA<sup>inhib</sup> : 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 neurons activity in stabilized colonies. **(B)** Left panel: CNO treatment over 5 consecutive days leads to an increase in the number of wins in VTA<sup>inh</sup> 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<sup>inh</sup> vs control  $*p<0.05$ . Right panel: CNO treatment leads to an increase in rank in VTA<sup>inh</sup> 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<sup>inh</sup> vs control  $**p<0.01$ ;  $***p<0.001$ . Error bars,  $\pm$  SEM. **(C)** Experimental design for decreasing VTA dopamine neurons 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<sup>inh</sup> (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<sup>inh</sup> mice won a number of 112 cumulated contests out of 162. 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<sup>inh</sup> mouse at the end of the stabilization. Chance level,  $p=0.17$  on 10000 simulations.