Dopamine promotes aggression in mice via ventral tegmental area to lateral septum projections

Darshini Mahadevia\textsuperscript{1,2,5}, Rinki Saha\textsuperscript{1,2,5}, Alessia Manganaro\textsuperscript{3}, Nao Chuhma\textsuperscript{2,4}, Annette Ziolkowski-Blake\textsuperscript{2}, Ashlea A. Morgan\textsuperscript{1,2}, Dani Dumitriu\textsuperscript{1,3}, Stephen Rayport\textsuperscript{2,4} & Mark S. Ansorge\textsuperscript{1,2} \textsuperscript{✉}

Septal-hypothalamic neuronal activity centrally mediates aggressive behavior and dopamine system hyperactivity is associated with elevated aggression. However, the causal role of dopamine in aggression and its target circuit mechanisms are largely unknown. To address this knowledge gap, we studied the modulatory role of the population- and projection-specific dopamine function in a murine model of aggressive behavior. We find that terminal activity of ventral tegmental area (VTA) dopaminergic neurons selectively projecting to the lateral septum (LS) is sufficient for promoting aggression and necessary for establishing baseline aggression. Within the LS, dopamine acts on D2-receptors to inhibit GABAergic neurons, and septal D2-signaling is necessary for VTA dopaminergic activity to promote aggression. Collectively, our data reveal a powerful modulatory influence of dopaminergic synaptic input on LS function and aggression, effectively linking the clinically pertinent hyper-dopaminergic model of aggression with the classic septal-hypothalamic aggression axis.
Agression is a complex social behavior that manifests across species from flies to humans\(^1\). It is defined as an overt act that can occur when the interest of two or more individuals conflict\(^2\). Classic lesion and stimulation studies have identified the septal-hypothalamic pathway as critical in controlling aggression\(^1\)–\(^6\). Specifically, stimulation of the ventral-lateral subdivision of the ventral-medial hypothalamus (VMHvl)\(^7\) and lesions of the LS both increase aggression across species\(^8\)–\(^11\). GABAergic neurons in the LS project to the VMHvl, thus establishing top-down inhibition in this septal-hypothalamic aggression axis. Optogenetic experiments in mice validated this model, demonstrating that activation of GABAergic LS neurons projecting to the VMHvl inhibit aggression\(^9\).

Monoamines are recognized for their capacity to modulate aggressive behavior\(^1\). While a subpopulation of serotonergic neurons that execute such influence has been identified\(^13\)–\(^14\), less is understood about the specific role of the dopaminergic neurons in controlling aggression. In humans, levels of dopamine (DA) metabolites in cerebrospinal fluid are positively correlated with psychopathic behavior\(^1\). Mutations in the gene coding for catechol-O-methyltransferase (COMT), responsible for the degradation of catecholamines including DA\(^16\), have been linked to aggression\(^17\). Furthermore, D2 receptor antagonists are currently used as first-line treatment in managing pathological aggression, indicating a causal role for high DA in aggression\(^18\),\(^19\).

In animals, the release of DA in the nucleus accumbens (NAC) is associated with increased aggression\(^20\). Mice lacking the dopamine transporter (DAT) exhibit a hyper-DAergic tone that correlates with increased aggression following mild social contact\(^21\). Furthermore, optogenetic activation of VTA DAergic neurons drives isolation-induced aggression\(^22\).

Building on this initial insight, here we investigated the modulatory role of population-specific and projection-specific DA function in mice, using optogenetics to manipulate DAergic neuronal activities and analyze behavior and post-synaptic electrophysiological responses. We furthermore paired retrograde tracing with brain-wide imaging analysis to map DAergic projection pathways. Our results identify a fundamental role for a subpopulation of VTA DAergic neurons that selectively project to the LS in orchestrating aggressive behavior, and highlight D2 receptors on GABAergic neurons in the LS as targets for drugs that ameliorate pathological aggression.

**Results**

Optogenetic activation of DAergic neurons in the VTA but not in the substantia nigra pars compacta promotes aggression. To begin to elucidate which DAergic neurons exert pro-aggressive influence over behavior, we optogenetically stimulated key DA circuit nodes and examined their role in behavior. First, we compared the effect of VTA versus substantia nigra pars compacta (SNc) DAergic neuron activation on aggressive behavior (Fig. 1a). For this purpose, we used DAT\(_{\text{irescre}}\); Ai32 mice, which conditionally express channelrhodopsin-2-EYFP (ChR2-EYFP) driven by the DAT promoter\(^23\),\(^24\). At 2 months of age, male mice were implanted with fiberoptic ferrules in the VTA or SNc. At 4 months of age, we stimulated and tested mice in isolation-induced aggression (Fig. 1a). We assessed aggression behavior in pairs of male mice where one mouse expressed ChR2 in DA neurons (carrying the DAT\(_{\text{irescre}}\) and one Ai32 allele), while the other mouse did not (carrying only the Ai32 or the DAT\(_{\text{irescre}}\) allele). In replication of our previous report\(^22\), we found that optogenetic stimulation (473 nm, 20 Hz, 10 ms pulse duration) of VTA DAergic neurons significantly increased aggression, as demonstrated by an increase in time spent fighting (Fig. 1b–d) and decrease in the latency to tail-rattle, mount, and bite (Supplementary Fig. 1a–c). Similar aggression patterns were observed against female mice (Supplementary Fig. 2a–e). Optogenetic stimulation of SNc DAergic neurons did not impact time (Fig. 1e–g) or latency (Supplementary Fig. 1d–f) measures of aggression, demonstrating a selective role for VTA DAergic neurons in promoting aggression.

Lateral septum and nucleus accumbens receive distinct DAergic projections from the VTA. DAT-positive VTA DAergic neurons project to the NAc, where increased DA release has been measured during aggressive behavior\(^11\). However, VTA DAergic neurons might also innervate other brain regions that are thought to modulate aggressive behavior, including the lateral septum (LS)\(^5\) and the VMHvl\(^7\). Using DAT\(_{\text{irescre}}\); Ai32 mice for axonal tracing, we found prominent DAergic input into the NAc and LS (Supplementary Fig. 3a, b), but not the VMHvl (Supplementary Fig. 3c). The pattern of innervation in the LS formed a crescent band, matching the distribution of tyrosine hydroxylase (TH) immunoreactivity\(^25\),\(^26\). We termed this anatomically defined septal region medial crescent lateral septum (MCLS). To investigate whether the DA projections in the MCLS originate from the VTA, we injected a retrograde Cre-dependent HSV virus conditionally expressing GCaMP (hEFlα-LSIL-GCaMP6s) into the LS (Fig. 2a). To anatomically compare the VTA DAergic neuronal population projecting to the LS with the population projecting to the NAc and to test for mutual collateralization, in separate mice we injected hEFlα-LSIL-GCaMP6s into the NAc (Fig. 2f). Four weeks after injection, brains were processed for double immunolabelling of TH and GFP (the latter to enhance the GFP component of the GCaMP molecule). Following brain-wide imaging (Supplementary Fig. 4a, b), we conducted a quantitative analysis of axonal projections and cell bodies, using the Wholebrain software\(^27\) registration function in RStudio and custom ImageJ macro scripts\(^28\). In LS injected animals, we detected a stream of retrogradely-labeled somas located in the VTA close to the midline (Fig. 2b, c, k, l) and in the periaqueductal gray (PAG) (Supplementary Fig. 10a–c and Supplementary Movie 1). The VTA-to-LS DAergic axonal projections formed a dense crescent structure in the MCLS (Fig. 2d). In NAc injected animals, we also detected a stream of retrogradely-labeled somas located in the VTA and PAG areas, but this stream was more lateral and less dense compared to LS-injected mice (Fig. 2g, h, k, l). Because GCaMP diffuses freely within the axonal lumen of neurons in which it recombines, we also assessed for collateralization. We did not detect GFP-positive axons in the LS of NAc injected mice (Fig. 2i), or in the NAc of LS injected mice (Fig. 2e), demonstrating that NAc and LS projecting DAergic neurons are different populations (Fig. 2o).

**DAergic input into the LS, but not the NAc, promotes aggression.** Next, we directly tested if DAergic projections to the LS or the NAc modulate aggression, with optogenetic stimulation in the MCLS or NAc in DAT\(_{\text{irescre}}\); Ai32 mice (Fig. 3a, f). We assessed aggression behavior in pairs of male mice where one mouse expressed ChR2 in DA neurons (carrying the DAT\(_{\text{irescre}}\) and two Ai32 alleles), while the other mouse did not (carrying only two Ai32 alleles). Optogenetic stimulation in the MCLS of ChR2 expressing mice (Supplementary Figs. 3b and 5) significantly increases time spent fighting (Fig. 3b, c) and decreased latency to attack (Supplementary Fig. 6a–c). Similar aggression patterns were observed against female mice as well (Supplementary Fig. 7a–e). Activation of DAergic terminals in the NAc (Supplementary Figs. 3a and 8) produced no significant difference in levels of aggression (Fig. 3g, h and Supplementary Fig. 6d–f). To investigate general motor activity, we stimulated both cohorts of mice in the open field and examined locomotor...
activity. We observed no increase in locomotor activity during optogenetic stimulation in either genotype in the MCLS cohort (Fig. 3d), while hyperactivity during optogenetic stimulation in ChR expressing mice was observed in the NAc cohort (Fig.3i).

Because DA plays a prominent role in motivation29, we next tested animals in real-time place preference. While the NAc cohort showed a significant preference for the side associated with stimulation as expected (Fig.3j), the LS cohort showed no real-time place preference (Fig.3e). Finally, to test for off-target effects by optogenetic light diffusing from the MCLS to the dorsolateral striatum (DS), we activated DAergic in the DS directly (Supplementary Figs. 3d and 9a) and saw no effect on aggressive behavior (Supplementary Fig. 9a–f). Together, these data reveal a dissociation between DAergic input to the NAc and LS and their respective roles in locomotion, reward, and aggressive behavior.

**Optogenetic activation of VTA-to-LS DAergic fibers increases aggression.** In the preceding experiment, we used DATIRESCre mice to conditionally drive ChR2 expression in the Ai32 line. This genetic strategy leads to the indelible expression of ChR2 in all DAT positive neurons. Indeed, we detected ChR2-positive cell bodies along an anterior-to-posterior stream ranging from the VTA to the PAG (Supplementary Fig. 10a–c). To specifically interrogate the VTA-to-LS pathway we adopted a viral approach: we injected male DAT cre mice with AAV5-ef1α-DIO-hChR2(H134R)-EYFP or AAV5-EF1a-DIO-EYFP control virus into the VTA to selectively express ChR2 in VTA DAergic neurons (Fig. 4b, c), and implanted a fiber optic ferrule above the MCLS (Fig. 4a and Supplementary Fig. 11a, b). Four weeks after viral injection and optic fiber implantation, animals were behaviorally tested. The same optogenetic stimulation (473 nm, 20 Hz, 10 ms pulse duration) in the MCLS significantly increased total fighting time (Fig.4d, e and Supplementary Fig. 12a–c). In the open field test, we detected a slight increase in locomotion during optogenetic stimulation (Fig.4f). In real-time place preference, we did not detect an effect of optogenetic stimulation (Fig.4g, h). Histological analysis confirmed no ChR2 or eArch expression in the PAG (Supplementary Fig. 13a–c), demonstrating that the viral
Fig. 2 Retrograde labeling of DAergic VTA→LS and VTA→NAc projection neurons. a, f Schematic diagram of retrograde hEF1α-LS1L-GCaMP6s injections into the LS and NAc of DATIRESCre mice. b, e, g, k Confocal images from hEF1α-LS1L-GCaMP6s injected DATIRESCre mice anti-GFP (green), anti-TH (red), and DAPI, (blue). b, g Whole section images containing the VTA. c, h Zoomed in confocal images of retrogradely labeled neurons in the VTA, arrows indicate double labeled cells. d Colocalization of GFP positive and TH positive fibers at the LS injection site. e Absence of GFP positive fibers in the LS of NAc injected mice. i Absence of GFP positive fibers in the LS of NAc injected mice. j Colocalization of GFP positive and TH positive fibers at the NAc injection site. k 2D reconstruction of the aggregate retrogradely labeled VTA-to-LS neurons (magenta) and VTA-to-NAc neurons (green) from four mice injected into the LS and four mice injected into the NAc. Soma locations were superimposed on one DAPI coronal image and overlaid with the outlines (white dashed lines) of the VTA brain areas from the Allen Mouse Brain Atlas. I VTA soma counts for LS and NAc injected animals. m, n Schematic of VTA-to-LS and VTA-to-NAc projection pathways. Aggregate of segmented somas (m) and terminals (n) for VTA→LS neurons (magenta) and VTA→NAc neurons (green). Coronal cartoon prepared based on Allen Brain Atlas. o Axonal projection density for LS and NAc injected brains, normalized by density at injection site. Scale bars: 300 μm (b, g), 200 μm (d, e, i, j), 50 μm (c, h), 1 mm (k). Quantification presented in mean ± SEM; n = 4 in each group.

Fig. 3 DAergic projections to the LS, not NAc, promote aggression. a, f Schematic diagrams for terminal stimulation in vivo. b, c Heightened aggressive behavior was observed in pairs when DATIRESCre; Ai32 mutant mice were stimulated (blue) in LS DA terminals. No effect of LS stimulation was detected in locomotor activity in the d open field or e real-time place preference (RTPP). g, h Aggressive behavior was not altered with NAc DA terminal stimulation (blue). i DA terminal stimulation in the NAc increased locomotor activity in the open field, during alternating 3 min off and on bouts of 20 Hz stimulation only in DATIRESCre; Ai32 mutant mice. j The percentage of the time, over a 20 min session, spent in the stimulated zone during RTPP was significantly increased following stimulation in mutants. Also shown representative heat maps of the time spent in the stimulated zone of the chamber during RTPP. **P < 0.01; ***P < 0.001 compared with their respective controls; mean ± SEM; n = 16 pairs (b, e), 8–9/group (d), 12/group (e), 20 pairs (g, h) and 20/group (i, j), box plots represent median and IQR and whiskers extend to maximum and minimum values (d, i).
approach indeed specifically targets the DAergic VTA-to-LS pathway. Together these data demonstrate that neurotransmitter release from axon terminals of VTA-to-LS projecting neurons is sufficient to increase aggression without altering DA mediated reward preference.

Axonal DA release in the LS induces inhibitory postsynaptic potentials in LS GABAergic neurons via D2 receptor activation. In the LS, 85% of the neurons are GABAergic, and Drd2 expression is higher than Drd1 expression. We therefore hypothesized that DA would act to hyperpolarize LS GABAergic projection neurons via D2 receptors and thereby reduce downstream GABAergic tone. To test this hypothesis, we first evaluated Drd2 and GAD1 expression in the LS using RNAscope in situ hybridization. In GAD1 positive cells, we found 82% colocalization with Drd2, and 95% of Drd2 positive cells were also GAD1 positive (Fig. 5a, b and Supplementary Fig. 14a). To evaluate DA neuron signaling to LS neurons, we next recorded from pyramidal-shaped neurons (located dorsal of the fornix) in acute brain slice preparations of the LS from DATIRESCre; Ai32 mice. Many neurons were not spontaneously active (12 among 61 neurons were active; 3.3 ± 0.7 Hz) (Fig. 5c, left), but depolarizing current injection (n = 14 cells, 50 pA) caused firing (burst firing: n = 11 cells, tonic firing: n = 3 cells) (Fig. 5c, right). This finding suggests that firing of the LS neurons in vivo is mostly driven by excitatory synaptic inputs, not by intrinsic pacemaker firing.

Fig. 4 Optogenetic activation of VTA→LS DAergic terminals leads to increase aggression. a Schematic diagrams for viral injection in DATIRESCre and terminal blue light stimulation in vivo. b Representative images of cell type-specific AAV-DIO-ChR2-eYFP expression (green) in VTA DA neurons (red), with colocalized neurons in yellow. Scale bar, 100 μm. c Quantification showed highly significant ChR2 expression in the TH+ cell. d, e The increased aggressive response was observed in pairs when DATIRESCre mice were stimulated (blue) in LS DA terminals. f LS DAergic terminal stimulation increased locomotor activity in the open field, during alternating 3 min off and on bouts of 20 Hz stimulation only in DATIRESCre ChR2 expressing mice. Vertical blue shading indicates optogenetic activation of ChR2. g During the RTPP task, percentage of time, over a 20 min session, spent in the stimulated zone was not significantly different in EYFP and ChR2 expressing mice. h The same result also displayed in the representative heat maps of the time spent in the stimulated zone of the chamber during RTPP. P < 0.01; ***P < 0.001 compared with their respective controls; mean ± SEM; n = 15 pairs, Box plots represent median and IQR and whiskers extend to maximum and minimum values (f). (Supplementary Data 1 for detailed statistics).
Optogenetic train stimulation (five pulses at 20 Hz) of EYFP-labeled DA neuron terminals elicited inhibitory postsynaptic responses (IPSPs) in 40 out of 61 recorded neurons. Among 40 cells, four cells were spontaneously active. In all of these four cells and two cells with induced firing by current injection, the firing was significantly reduced by the IPSPs (Supplementary Fig. 14b). The IPSPs were blocked by the D2 receptor antagonist sulpiride (Fig. 5d–f), suggesting that these sub-second IPSPs were mediated by G-protein coupled inward rectifying K⁺ channels directly coupled with D2 receptors, as observed in the ventral midbrain DA neurons and in striatal cholinergic interneurons. Lastly, we detected a very small EPSP that was CNQX sensitive, indicating glutamate co-transmission.

VTA-to-LS DA signaling is necessary for normal aggression. To investigate if DAergic input from the VTA into the LS is necessary to drive normal aggressive behavior we performed an optogenetic inhibition experiment, using archaerhodopsin, a light-gated proton pump which inhibits neural activity upon photostimulation. Specifically, we bilaterally injected male DATIRIReScre mice with AAV5-EF1a-DIO-eArch3 or AAV5-EF1a-DIO-EYFP control virus into the VTA and implanted fiber optic ferrules above the MCLS (Fig. 6a–c and Supplementary Fig. 15a, b). Four weeks after viral injection and fiber implantation we tested mice in social isolation-induced aggression. Optogenetic stimulation with green light (532 nm, 10 mW, continuous 55 s) of DAergic fibers in MCLS in eArch injected animals during a fight bout reduced aggression (Fig. 6d). Overall, total fight time significantly decreased (Fig. 6e and Supplementary Fig. 16a–c). Inhibitory opsins can have excitatory side effects. Such effects have largely been reported for extended opsin activation for more than 1 min, which is the reason why we had limited our activation protocol to 55 s. Furthermore, excitatory effects are mostly seen after but not during opsin activation (rebound effect and increased spontaneous release). Hence, in order to further minimize the risk that excitatory side effects might account for our behavioral effect, we limited the behavioral analysis to only the first aggression bout and to only the 55 s of light application. Impressively, we find close to a complete block...
of aggressive behavior using this refined analysis (Fig. 6f, g). Locomotion in the open field test was not affected (Fig. 6h). Furthermore, optogenetic inhibition did not change behavior in the real-time place preference task (Fig. 6i, j). Together these data demonstrate that VTA-to-LS DAergic projections are necessary to establish normal aggression levels but play no role in normal locomotion or real time place preference.

D2 signaling within the LS is necessary for VTA DAergic neurons to promote aggression. TA projections to the LS might constitute the dominant pathway involved in DAergic facilitation of aggressive behavior. Alternatively, VTA DAergic projections to other brain regions might also contribute to DAergic facilitation of aggressive behavior. To evaluate the necessity of the LS pathway for VTA-triggered aggression, we infused sulpiride or vehicle into the LS (Supplementary Fig. 17), followed by VTA DAergic neuron activation during aggression testing (Fig. 7a). We found that D2 antagonism in the LS completely blocked VTA-triggered aggression (Fig. 7b, d–g), without altering general locomotor activity (Fig. 7c and Supplementary Fig. 18a–c). These data demonstrate that the VTA-to-LS DAergic pathway, via D2 activation on GABAergic LS neurons, is necessary to drive VTA-triggered aggression.
Fig. 7 D2 receptor activation in the LS is necessary for VTA DAergic activity to increase aggression. a The schematic diagram for local infusions of sulpiride in the LS followed by optogenetic stimulation (blue) of the VTA to evoke in vivo non-projection specific DA release. b Local sulpiride infusion into the LS prevents aggression elicited by optogenetic activation of VTA DAergic activity. c No difference in locomotion was detected between the groups following local infusion of sulpiride in the LS. Time-fighting, for individual samples, is broken down by d, e treatment and f, g stimulation condition. *P < 0.05; ***P < 0.001 compared with their respective controls; mean ± SEM; n = 11 pairs (b, d–g) and 20/group (c). VEH, vehicle.

Discussion

We identified a role for DAergic VTA-to-LS projecting neurons in aggression. We demonstrate that DAergic neurons densely innervate a subregion within the LS, which we termed MCLS (medial crescent lateral septum), where DA signaling is necessary and sufficient for orchestrating aggressive behavior.

The LS controls emotionality and aggression. Several inputs into the LS have been identified that modulate these behavioral domains. For example, ventral hippocampus neurons projecting from CA3 to the LS play a major role in controlling anxiety and contextual fear discrimination, while projections from CA2 to the LS regulate aggressive behavior. Our findings add DAergic innervation from the VTA not just as a component that is sufficient to increase aggressive behavior but as a necessary input under baseline conditions to establish normal aggression levels. We find that terminal neurotransmitter release from DAergic axons in the LS inhibits GABAergic LS neurons via postsynaptic D2 receptor activation. A more detailed topographical analysis of PSC responses, as we have for example done in the striatum and not-abundant DA neuron inputs limited detection of PSCs in slice preparation. Thus, while we were able to qualitatively characterize identified PSCs, we cannot conclude on the precise quantitative aspect nor the topographical distribution for gradients in connectivity. Furthermore, how coordinated inputs from the VTA and other regions such as the hippocampus interact to control LS output and whether DA release in the LS also modulates inputs at the presynaptic levels remains to be investigated.

We find a dissociation in the behavioral roles of DAergic projections to the MCLS and NAc, where MCLS input drives aggression while NAc input drives reward and motor activity. This finding is in line with the functional organization of monoamine systems, where relatively few monoaminergic neurons reside as densely packed clusters in the midbrain and brainstem but project widely to distributed target areas, modulating behaviors that are controlled by multiple target regions. The result of manipulating DAergic activity is thus greatly dependent on which specific DAergic target site is being manipulated. Our data indicate that separate DAergic pathways drive distinct behavioral components of aggression. While NAc DA does not initiate aggression, it might ascribe positive valence to LS-triggered fighting behavior and thereby contribute to proactive aggression.

Mirroring the dissociation at the behavioral level, we also find a dissociation at the anatomical level. Specifically, we identified VTA DAergic neurons that innervate the MCLS as a unique neuronal population, that is different from DAergic neurons that project to the NAc. Cell bodies reside at distinct locations within the VTA and do not co-innervate each other’s target region. DAergic fiber distribution within the LS is in line with TH immunoreactivity in the LS of rats and mice. Neurons projecting to the LS are concentrated in a relatively more anterior and medial location (Supplementary Movie 1) within the VTA, than those projecting to the NAc.

The absence of immunohistochemical evidence of collateralization comports with each target site controlling distinct functional outputs. Future studies of genetic and molecular markers in MCLS-projecting and NAc-projecting cell populations might help to better understand their unique properties and their ontogeny. Given the known association between NAc DA release and aggression as well as the necessary role for D2 expressing NAc neurons in the rewarding properties of aggression, it will...
be important to understand the coordinated activities of both cell populations during behavior. Using fiber photometry to monitor Ca\(^{2+}\) transients with genetically encoded fluorescent Ca\(^{2+}\) sensors in projection specific DAergic neuronal populations would for example reveal their real time activity by proxy of CA2+ transients\(^ {65,67}\). Alternatively, using fiber photometry to monitor DA release with genetically encoded fluorescent DA sensors in DAergic projection target regions would reveal their real time activity by proxy of extracellular DA content\(^ {68}\). Using these techniques to test whether and how the LS-projecting and NAc-projecting VTA DA neurons change their activity pattern during the natural aggression behavior will be important to better understand the coding properties of both pathways. While we hypothesize that the VTA(DA)-to-LS pathway is important for the initiation and maintenance of fighting behavior while the VTA(DA)-to-NAc pathway assigns valence and establishes reinforcing properties to aggressive behavior, this hypothesis can only be properly scrutinized by analyzing neuronal activities during behavior. Future work will directly assess such temporal interplay between spatially segregated DAergic pathways in complex and dynamic behavior.

Our identification of a population-specific and projection-specific DAergic input to the LS that exerts a powerful influence over aggressive behavior effectively join two major streams of aggression research—the hyper-DAergic model of aggression\(^ {12,49}\) and the classic LS-hypothalamic aggression axis involving “septal rage”\(^ {56}\) and the “hypothalamic attack area”\(^ {7}\). Psychological and behavioral data furthermore support the classification of aggression into two dominant forms: (1) reactive aggression which occurs impulsively in response to perceived external threat and (2) proactive aggression that is premeditated and directly motivated by a drive for appetitive reward\(^ {50}\). VTA DAergic activity is prominently involved in reward coding\(^ {51}\). Hence, one might expect a role for VTA-to-LS input in proactive aggression\(^ {50,52,53}\). However, our findings of DAergic LS input promoting aggression without affecting real time place preference indicate a role for DA in reactive aggression\(^ {54}\). Extreme forms of impulsive aggression are directed against the opposite sex\(^ {45}\). Indeed, we find that male mice also attack females when the VTA-to-LS input is stimulated. Aggressive, but not sexual behavior towards females\(^ {55,56}\), has been discussed as displaced aggression towards an inappropriate target\(^ {57}\). This description is reminiscent of an impulsive form of aggression seen in humans with intermittent explosive disorder, who display inappropriate and violent outbursts towards targets that don’t typically elicit aggression\(^ {50,58}\). The lack of reward coding together with indiscriminate targeting further implicates the VTA-LS hyper-DAergic aggression circuit in impulsive and reactive aggression, rather than outcome-oriented and proactive aggression.

Our findings suggest that genetic and environmental factors that increase LS DAergic activity can be risk factors for aggression-related psychopathology. Acute and repeated exposure to DA-boosting drugs such as psychostimulants can increase aggression\(^ {59}\). In mice, transient increase of DA signaling during adolescence can even permanently increase aggression throughout life\(^ {25}\). Also, genetic variants, that impact DAergic activity, can increase baseline aggression and thereby contribute to clinical psychopathy. For example, the low-activity \textit{COMT} (V158M) allele is associated with aggressive personality traits\(^ {60}\), and the 10-repeate allele of \textit{DAT1} is linked to aggressive and antisocial dispositions\(^ {61}\). Our data indicate that such genetic risk might act via increasing (re-) activity of the DAergic VTA-LS pathway\(^ {12,22}\). Furthermore, our findings indicate that clinically established pharmacotherapy, currently used to manage pathological aggression, acts on D2 receptors within the LS to specifically reduce aggression, and not via DA receptor-mediated sedation\(^ {19,62}\). This insight underscores the probable utility of selectively targeting broadly expressed GPCRs on specific cell types\(^ {63}\). Together our data reveal DA circuit mechanisms underlying aggressive behavior in mice with potentially translational relevance for understanding normative and pathological aggression in humans.

**Methods**

**Animals.** All animal testing was conducted under protocols approved by Columbia University and New York State Psychiatric Institute Institutional Animal Care and Use Committee. Adult male mice (2–12 weeks) were used for all studies. Mice were housed at 18–23 °C, with 40–60% humidity under a 12 h light-dark cycle (lights on at 7 am), with food and water available ad libitum. DAT\(^ {\text{iresc}}\) and Ai32 (RCL-Chr2(H134R)/EYFP)\(^ {23}\) mice were crossed to produce experimental cohorts consisting of DAT\(^ {\text{iresc}}\), Ai32 and single mutant controls on a mixed F1 background (C57BL/6 J × 129S2/Jr/EiTac). DAT\(^ {\text{iresc}}\) Ai32 mice expressed ChR2 in >95% of DAergic neurons, based on immunohistochemistry\(^ {64}\). Thus, this strategy allowed us to interrogate almost all of DAergic inputs in target regions without variability. For the RNAseq experiment, Drd2-EGFP, Gensat-S118Gsat/Mmnc mice were used. Given that female mice do not display territorial aggression, only male mice were investigated.

**Viruses.** AAVs\(^ {\text{ef1a-DIO-hChR2(H134R)-EYFP}}, AAV5-\text{EF1a-DIO-eArch3.0-EYFP},\) and AAV5-\text{Ef1a-DIO-EYFP} viruses were purchased from the University of North Carolina UNC Gene Therapy Vector Core. All AAV titers ranged from 3.00 × 1012 to 4.30 × 1013 genomic copies per ml. HSV titer was 1.00 × 109 genomic copies per ml.

**Stereotoxic surgery and viral vectors.** All behavioral mice were implanted with optical fiber ferrules (Ceramic ferrules (OD 1.2 mm)): Precision fiber products; Optical fiber (OD 0.2 mm): Thorlabs, Newton, NJ, USA). During surgery, mice were anesthetized with ketamine (10 mg/kg body weight) and xylazine (50 mg/kg body weight) and a stereo dissecting microscope and a binocular microscope (30 µg/kg) or isoflurane (1–1.5%) solution and placed in a stereotactic frame. For genetic approach optogenetic stimulation experiments, the optical fiber ferrules were implanted over the VTA (AP: −3.5; ML: ±0.5; DV: −4.2), the SNc (AP: −3.5; ML: ±1.6; DV: −4.4), the LS (AP: 0.6; ML: ±0.4; DV: −2.8) and the NAc (AP: 1.6; ML: ±1.4; DV: −3.8).

For the optogenetic activation experiment of VTA-LS, 500 nl of AAV5-\text{ef1a-DIO-hChR2(H134R)-EYFP} or AAV5-\text{Ef1a-DIO-EYFP} was injected ipsilaterally into VTA and optical fiber ferrule was implanted over LS in the same hemisphere. For the optogenetic inhibition experiment of VTA\(^ {\text{\text{95}}}\), 500 nl of AAV5-\text{EF1a-DIO-eArch3.0} or AAV5-\text{Ef1a-DIO-EYFP} was injected bilaterally into VTA and optical fiber ferrule was implanted over LS in both hemispheres.

For the retrograde labeling experiment, 200 nl of HFI-LS1L-GCaMP6s was injected ipsilaterally into LS and NAc, respectively. Brains were collected 4 weeks later.

For the local infusion experiments, DAT\(^ {\text{iresc}}\) Ai32 mice were unilaterally implanted with a guide cannula (26 gauge; PlasticOne) directly above the LS (AP: +0.6 mm, ML: −0.4 mm, DV: −2.3 mm).

**Behavioral testing.** To assess aggressive behavior, we used the isolation-induced aggression paradigm\(^ {65}\). The home cage was divided in half by a perforated partition made of clear plastic, pairing DAT\(^ {\text{iresc}}\), Ai32 mutant mice with controls. Mice were housed for 10 days before the experiment was performed. For the optogenetic activation experiment, on test day, dividers were taken out and blue light pulses (473 nm, 20 Hz, 10 ms pulse duration) were delivered to the region being investigated for 30 s. The time spent fighting was scored as a sum of the time spent biting, tail rattle, and mounting. For the optogenetic inhibition experiment, on the test day, when the fighting bout started, the mouse of the pair were stimulated with continuous green light (532 nm, 10 mW) for 55 s in the lateral septum.

The assay was moderately adapted to investigate the effect of local infusions of D2 receptor antagonist, sulpiride, in the LS. Fifteen minutes before the test, mice received 0.13 µl volume infusions of either sulpiride (Sigma Aldrich; 0.05 µg/µl) or vehicle (0% physiological saline) microinjected at a rate of 0.05 µl/min over 3 min. The injection cannula (28 gauge; PlasticOne) was left in place for 2 min after the infusion and the mice were returned to their home cages before aggression testing using the stimulation protocol described above.

Locomotor activity in an open field, in response to optogenetic activation, was assessed in PleaGlas activity chambers as previously described\(^ {66}\). The open field is a standard test for locomotor behavior. It consists of a simple square enclosure that is equipped with infrared detectors to track animal movement in the horizontal and vertical planes. Measures of total distance covered during locomotion are used as an index of activity. Mice were placed into the center of the open field and activity was recorded for 12 min in 3 min bouts of alternating light-off and light-on conditions. Testing took place under bright ambient light conditions and the total distance traveled was measured.
Histology. Animals were deeply anesthetized with ketamine/xylazine and perfused with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde (Electron microscopy sciences, Cat# 1574-5) in PBS. Brains were then removed and post-fixed for 3 h in 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were then dehydrated in 10, 20, 30% sucrose and frozen. Coronal sections (50 µm thick) were cut either with a cryostat (Leica Biosystems) or vibratome (Leica Biosystems) and fluorescence immunohistochemistry was performed on free-floating sections.

Immunohistochemistry. Sections were first rinsed with PBS and then blocked with buffer (PBS, 5% normal goat serum (NGS), 5% normal donkey serum, and 0.5% Triton X-100). This was followed by overnight incubation with primary antibody and every section was placed to a fluorophore-labeled secondary antibody in the body. The sections were incubated in primary antibody for 24 h at room temperature, washed 3x with PBS, and then incubated with secondary antibody for 1 h at room temperature. After washing 3x with PBS the sections were incubated with DAPI (1:20,000; catalog no. D1306, Invitrogen) and transferred into PBS solution.

Fluorescent in situ hybridization with RNAscope®. The expression and colorization of dopamine receptor 2 (Drd2), glutamate decarboxylase (GAD1), in mutant mice were verified using an in-situ hybridization assay. Drd2-EGFP mice were culled by cervical dislocation and the brains removed from the skull and snap-frozen. The vibratome sliced dissected sections were immersed in the cryoprotectant mixture (Fisher Scientific; catalog no.12-550-15) and cover slipped with Prolong Gold Antifade (catalog no. P36930; Invitrogen).

For optic fiber location identification (Supplementary Figs. 3a–d, 5, 8, and 17) GFP, immunohistochemistry was performed using a rabbit primary antibody against GFP in the in the developing 16 section 50 µm coronal brain sections that include the lateral septum area using a cryostat (CM3050S Leica Biosystem) and thaw-mounted onto Superfrost Plus Microscope Slides (Fisher Scientific; catalog number 12-550-15). The samples were prepared and pretreated for the assay using the RNAscope® Sample Preparation and Pretreatment Guide for Fresh Frozen Tissue. The RNAscope® Multiplex Reagent kit (Advanced Cell Diagnostics, catalog number 320293) was used to identify the manufacturer’s instructions. Sections were hybridized with D2 or GAD1 probes. The HybEZTM oven (Advanced Cell Diagnostics, PN 321710/321720) was used in the heating steps and the slides were mounted using Prolong Gold Antifade (Thermo Fisher Scientific; catalog no. P36930). Images were acquired using a laser confocal scanning microscope (Leica, TCS SP8) and positive labeling was counted using LAXx software (Leica, TCS SP8).

Slice electrophysiology. DAPT;B6C3F1;Alb2 mutant mice (P72-93) were anesthetized with a ketamine (90 mg/kg)/xylazine (7 mg/kg) mixture. After confirmation of brain reactivity and integrity, mice brains were immersed in ice-cold high glucose artificial cerebrospinal fluid (ACSF) (in mM: 75 NaCl, 2.5 KCl, 26 NaHCO3, 1.25 NaH2PO4, 0.7 CaCl2, 2 MgCl2 and 100 glucose, pH 7.4) saturated with carbogen (95% O2 and 5% CO2). Coronal sections of the septum (300 µm, 0.1 mm anterior to bregma) were made with a vibrating microtome (VT1200S, Leica). Sections were incubated in high glucose ACSF at room temperature for at least 1 h for recovery, then a slice was transferred to the recording chamber (submerged, 500 µl volume) on the stage of an upright microscope (BX61WI, Olympus), continuously perfused with standard ACSF (in mM: 125 NaCl, 2.5 KCl, 25 NaHCO3, 1.25 NaH2PO4, 2 CaCl2, 1 MgCl2, 100 glucose, pH 7.4) saturated with carbogen. Neurons were visualized using enhanced bright light interference contrast (DIC) optics with a scientific CMOS camera (ORCA-Flash4.0LT, Hamamatsu photonics). Recording patch pipettes were fabricated from standard-wall borosilicate glass capillary with filament (World Precision Instruments). Pipette resistance was 3–7 MΩ. Composition of intracellular solution for both whole-cell and patch-clamp recordings was 4 M potassium choline chloride (KCl; ≥99%), 130 M potassium methylsulfate (MeSO4), 5 KCl, 2 MgCl2, 0.1 CaCl2, 10 HEPES, 1 EGTA, 2 ATP and 0.1 GTP, pH 7.25. Fast current-clamp recording was performed with an Axopatch 200B amplifier (Molecular Devices). Liquid junction potential (~10 mV) was adjusted online. Series resistance was 15–27 MΩ and was not compensated. The recording was performed in the L5 just dorsal to the fornix, in areas with high EJF fluorescence of DA neuron terminals, from large pyramidal-shaped/triangular-shaped neurons located. One to two slices per animal included the area innervated by DA neurons, and only one slice includes sufficient amount of DA fiber innervation to evoke synaptic responses. In some animals, neither of the slices had sufficient density of DA fiber innervation, and those slices were discarded. Synaptic responses were evoked by 5 ms duration field illumination with a high-power blue LED (Thorlabs) delivered in trains of five pulses at 20 Hz, repeated at 1 min intervals. For pharmacological studies, drugs were delivered by perfusion. Recordings were done at 31–33 °C (TC 344B Temperature Controller, Warner Instruments). The intracellular solution data were recorded at 5 kHz (Pclamp 10, Molecular Devices), filtered at 5 kHz with a 4-pole Bessel filter, and recorded using pClamp 10 (Molecular Devices). Data were analyzed with Axograph X (Axograph Scientific).

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Average firing frequencies were calculated in 0.5 s windows: for baseline, 0.5–1 s prior to the onset of a training stimulus, and for stimulus effects, 0.1–0.6 s after the onset of the train. PSC peak amplitude was measured in averages of three consecutive traces.

**Statistical analysis.** Statistical analysis for behavior was performed using StatView 5.0 software (SAS Institute, Cary, NC, USA) or Prism9 software (GraphPad) and Microsoft Excel. Data were analyzed using Student’s t-test, one-way or two-way ANOVA with Student–Newman–Keuls post hoc testing as indicated. The criterion for significance for all analyses was $^*P<0.05; ^{**}P<0.01; ^{***}P<0.001$. For the physiology, statistical tests were done using SPSS 23 (IBM). Since sample sizes were small, for the independent sample $t$-test, we did not assume equal variance. Sample size estimation was done by G$^2$Power 3.1, with $P=0.05$, power $=0.9$. The effect size was estimated from previous experiments. Results are expressed as mean ± SEM.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Code availability**  
Associated Code can be found here: https://github.com/albilard/Neuro_quantify_projection.

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Author contributions

D.M. and R.S. performed all behavioral, anatomical, and confocal microscopy experiments, N.C. performed electrophysiological experiments, A.Z. assisted with behavior, histology, RNAscope experiment, A.A.M. helped in imaging experiments, A.M. wrote custom image analysis scripts and conducted the quantification of brain-wide anatomical data, D.D. supervised the anatomical quantification S.R. supervised electrophysiological experiments, M.S.A. supervised the study. D.M., R.S., N.C., S.R., and M.S.A. wrote the manuscript. Both D.M. and R.S. contributed equally and have the right to list their name first in their CV. All authors contributed to the article and approved the submitted version.

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to Mark S. Ansorge.

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