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GABAergic miR-34a regulates Dorsal Raphè inhibitory transmission in response to aversive, but not rewarding, stimuli

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The brain employs distinct circuitries to encode positive and negative valence stimuli, and dysfunctions of these neuronal circuits have a key role in the etiopathogenesis of many psychiatric disorders. The Dorsal Raphè Nucleus (DRN) is involved in various behaviors and drives the emotional response to rewarding and aversive experiences. Whether specific subpopulations of neurons within the DRN encode these behaviors with different valence is still unknown. Notably, microRNA expression in the mammalian brain is characterized by tissue and neuronal specificity, suggesting that it might play a role in cell and circuit functionality. However, this specificity has not been fully exploited. Here, we demonstrate that microRNA-34a (miR-34a) is selectively expressed in a subpopulation of GABAergic neurons of the ventrolateral DRN. Moreover, we report that acute exposure to both aversive (restraint stress) and rewarding (chocolate) stimuli reduces GABA release in the DRN, an effect prevented by the inactivation of DRN miR-34a or its genetic deletion in GABAergic neurons in aversive but not rewarding conditions. Finally, miR-34a inhibition selectively reduced passive coping with severe stressors. These data support a role of miR-34a in regulating GABAergic neurotransmitter activity and behavior in a context-dependent manner and suggest that microRNAs could represent a functional signature of specific neuronal subpopulations with valence-specific activity in the brain.

Dorsal Raphè | GABA | microRNA | stress

Significance

The Dorsal Raphè Nucleus (DRN) is an important source of neurotransmitters in the brain that is involved in valence encoding. Recent studies have highlighted that specific subpopulations of neurons within a brain area encode positive or negative stimuli. The functional dissection of such circuits is a relevant goal both in neuroscience and in psychiatry. Here, we found that miR-34a is selectively expressed in ventrolateral DRN GABAergic neurons. Moreover, we report that this molecule has a selective functional role in the regulation of inhibitory transmission. Specifically, miR-34a regulates DRN GABAergic activity and behavior in response to aversive, but not rewarding, experiences. These data also provide evidence that microRNAs could represent a potential tool for functional dissection of brain circuits.

Processing of rewarding and aversive experiences is fundamental for survival. Experiences characterized by positive and negative valence require the expression of different behavioral strategies supported by the engagement of specific neuronal circuits. Functioning of these circuits guarantees survival and health while their dysfunction can cause both physical and mental disorders.

The Dorsal Raphé Nucleus (DRN) is an essential source of neuromodulators and is involved in a wide range of behaviors, including the response to rewarding (1–3) and aversive stimuli (4–7). The DRN processes these behaviors thanks to its anatomical and cellular complexity, characterized by functionally distinct subregions organized in multiple cell types (8–10). Indeed, specific subpopulations of neurons within the DRN selectively respond to rewarding or aversive stimuli (6, 11, 12). Previous studies have mostly focused on serotonin (5-HT) neurotransmission because the DRN is the primary source of serotonergic innervation of the brain. However, local γ-aminobutyric acid (GABA) signaling also plays a crucial role in the physiology of this nucleus (13) by modulating serotonergic neuron excitability and 5-HT release in several brain areas (13–15). Studies in rodents have demonstrated that DRN GABAergic transmission mediates responses to aversive and rewarding stimuli (2, 4, 16, 17). Indeed, DRN neurons process different rewards including sucrose, food, sex, and social interactions. When mice consume freely available food or sucrose, DRN 5-HT neurons are activated while GABAergic neurons are inhibited (2). DRN neurons are also engaged in response to aversive experiences; however, their pattern of response depends on the expressed behavior and on the intensity of the negative experience (4). These findings support the involvement of distinct neuronal populations in mediating behavioral responses with different valence. Despite its potential interest in the context of psychiatric disorders, the functional segregation of GABAergic neurons within these local inhibitory circuits has not been elucidated.

Specific patterns of transcriptional profiles drive the identity and physiology of functionally distinct subneuronal populations. In this context, microRNAs (miRNAs) may have a pivotal role, as miRNA expression is often specific at neuronal and subneuronal levels (18–20). Notably, single-cell RNA sequencing data in mice have revealed the expression of a subset of miRNAs with distinct profiles in glutamatergic and GABAergic neurons and subtypes of GABAergic neurons (21).

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Previous work in the DRN has shown a key role of miRNAs in the physiology and pathophysiology of the DRN (22–26). We recently provided evidence of a highly selective expression of miR-34a in this nucleus, where it is required for the behavioral response to stress (23, 24) as well as for stress-induced 5-HT release in the medial prefrontal cortex (mPFC). Indeed, miR-34a, which belongs to the miR-34 family together with miR-34b and miR-34c, is known to be involved in neurotransmitter release (23, 24, 27–29), likely at dendritic level (23, 30).

In the present study, we tested the hypothesis that expression of miR-34a modulates activity of GABAergic neurons in the DRN of mice exposed to motivationally salient conditions. To this end, we combined in situ hybridization (ISH), miRNAscope ISH, and immunofluorescence to identify the regional and cellular selectivity of miR-34a expression in the mouse brain. Moreover, we tested whether GABAergic miR-34a regulates responses to aversive and rewarding experiences using pharmacological and genetic strategies.

Results

miR-34a Is Selectively Expressed in GABAergic Neurons of the Ventrolateral DRN. We have previously shown that miR-34a is highly expressed in the DRN (23, 24). Here, we further characterized miR-34a localization by ISH and showed that this miR is exclusively expressed in the ventrolateral part of the DRN (Fig. 1 *A*, *i* and *SI Appendix*, Fig. S1). With a combined miRNAscope and immunofluorescence approach, we confirmed the selective localization of miR-34a in the ventrolateral part of the DRN. Furthermore, colocalization analysis showed that miR-34a is present exclusively in GAD67-positive (+) neurons, while it is lacking in 5-HT+ neurons (t = 31.33; df = 5; $P = 0.0001$, Fig. 1 *C* and *D*). Moreover, we found that ~67% of GAD67+ cells in the DRN expressed miR-34a and ~33% did not (Fig. 1 *C*, *ii* and *D*), suggesting that this miR is expressed only in a subpopulation of GABAergic neurons. The specificity of the miR-34a probe was confirmed by performing a miRNAscope ISH assay on brain slices

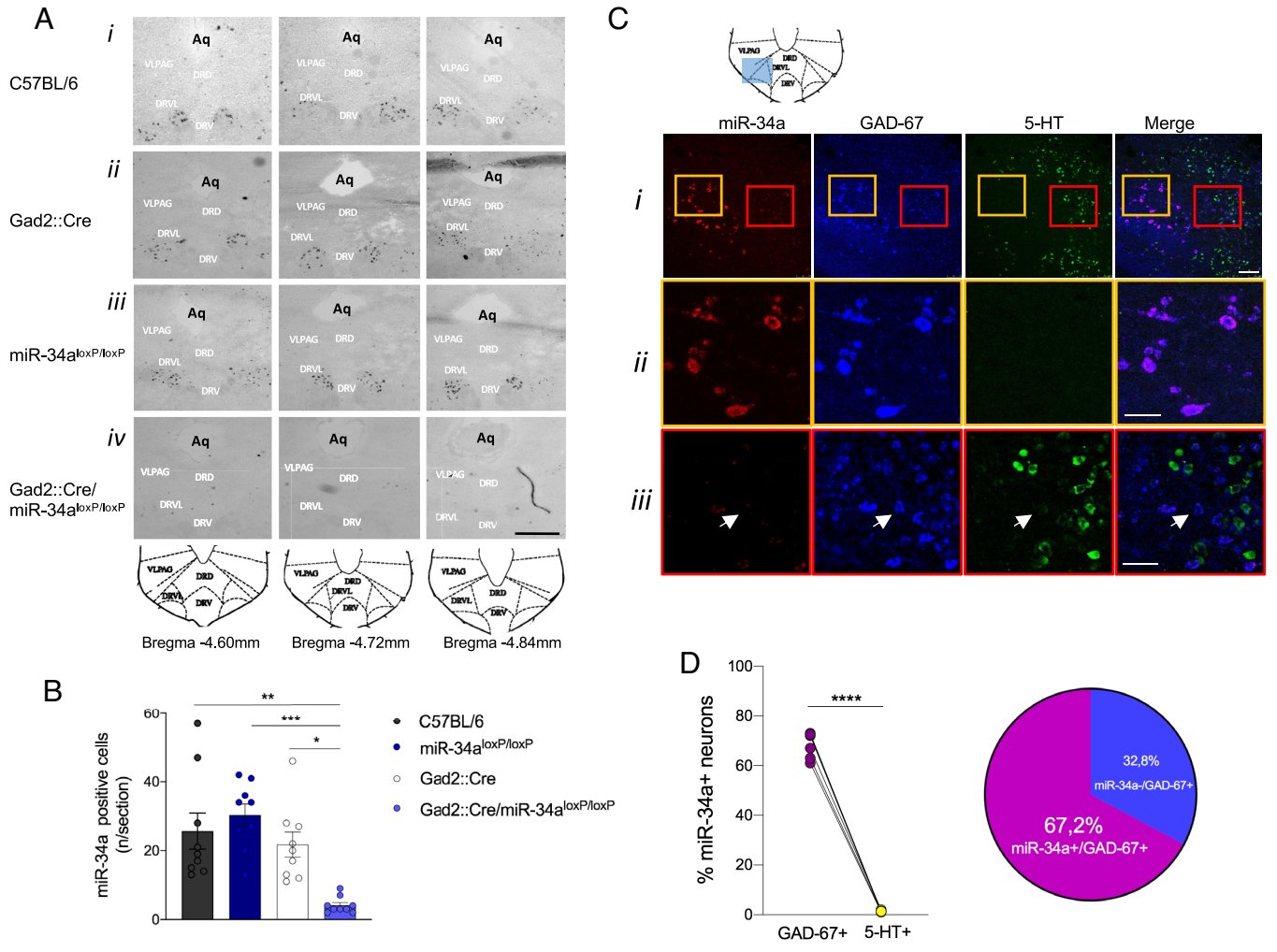

Fig. 1. miR-34a is selectively expressed on GABAergic neurons of the Dorsal Raphè Ventrolateral Nucleus (DRVL). (*A*) Three representative coronal brain sections (along the rostrocaudal axis) of C57BL/6 (*i*), Gad2::Cre (*ii*), miR-34$^{loxP/loxP}$ (*iii*), and Gad2::Cre (*iv*) mice showing the miR-34a ISH signal in the DRN. (Scale bar, 500 µm.) (*B*) mean ± SEM of the number of miR-34a positive cells in the DRN of the different experimental groups showing a significant decrease of miR-34a positive cells in Gad2::Cre/miR-34$^{loxP/loxP}$ mice compared to C57BL/6, Gad2::Cre, and miR-34$^{loxP/loxP}$ mice (N = 3 animals per group, 3 sections/animal). *$P < 0.05$, **$P < 0.001$, ***$P < 0.0001$. (*C*) Representative images of miRNAscope ISH combined with immunofluorescence performed on coronal brain sections at the level of the DRN region in C57BL/6 mice. Columns: low- and high-magnification images of a coronal brain section. Rows: triple-labeled confocal images of the DRN, with miR-34a (red), serotonin (5-HT, green), GAD67 (blue) and merged signals. *i*) low-magnification images, *ii*) example of GAD-67+/miR-34a+ colocalization, *iii*) example of GAD-67+/miR-34acolocalization. Scale bars: (*i*) low-magnification 100 µm; (*ii* and *iii*) high-magnification 40 µm. (*D*) *Left* panel: Percentage of miR-34a that colocalized with GAD-67 and 5-HT neurons in the DRN of C57BL/6 mice (N = 3 per group, 2 sections/animal). *Right* panel: Pie chart reporting the percentage of GAD-67 cells expressing miR-34a. DRVL; Dorsal Raphe Dorsal (DRD); Dorsal Raphè Ventral (DRV).

obtained from miR-34a knock-out mice: in these slices, no miR-34a signal was detected in the DRN (*SI Appendix*, Fig. S2).

Finally, to further demonstrate the selectivity of miR-34a expression in GABAergic neurons of the DRN, we employed a genetic strategy. Thus, the miR-34a gene was selectively deleted on GABAergic neurons and residual miR-34a expression levels were evaluated. For this purpose, Gad2::Cre mice were crossed with miR-34$^{loxP/loxP}$ mice to obtain a mouse model lacking miR-34a selectively on Gad2 expressing neurons (Gad2::Cre/miR-34$^{loxP/loxP}$). Strikingly, ISH analysis revealed that in these mice DRN miR-34a was barely detectable [cell count: $F(3, 32) = 10.07$, $P = 0.0001$; Fig. 1 *A* and *B*].

Altogether, our results demonstrate that, in the DRN, miR-34a is selectively expressed in a subpopulation of GABAergic neurons.

Both Aversive and Rewarding Experiences Reduce GABAergic Transmission in the DRN. Prior to examining the role of miR-34a in DRN GABAergic neurotransmission, we evaluated the physiology of DRN GABA release in response to acute exposure (2 h) to an aversive or a rewarding experience in C57BL/6 mice. Exposure to chocolate was chosen as the rewarding experience because this stimulus is able to elicit an approaching and consummatory behavior in a standard feeding regime, as previously reported (31) and as confirmed by our data (*SI Appendix*, Fig. S3 *A* and *B*). Restraint stress was chosen as the negative stimulus since it is a severe and inescapable aversive condition. This type of experience has been demonstrated to induce a pattern of DRN GABAergic response that is similar to the one elicited by a reward (2, 4).

We used two complementary experimental settings (timelines in Fig. 2 *A* and *B*): 1. in-vivo microdialysis, through which we measured the levels of extracellular GABA in the DRN *during* the 2 h of experience with the stimulus; 2. ex-vivo electrophysiology, through which we evaluated miniature inhibitory postsynaptic currents (mIPSCs) of putative 5-HT neurons (see *Materials and Methods* for details) in DRN slices collected from mice exposed to the aversive or the rewarding experience.

Our results show that both restraint stress and chocolate exposure produced a significant time-dependent decrease of GABA outflow in the DRN, measured by in vivo microdialysis (main effect of time: $F(1, 14) = 6.352$, $P = 0.0002$; Fig. 2*C*). Specifically, in response to restraint stress, extracellular GABA showed a significant reduction at 60, 80, 100, and 120 min from the start of the protocol. The exposure to chocolate also significantly reduced extracellular GABA outflow in the DRN (time points: 20 to 120 min from the start of the protocol, Fig. 2*C*). Note that GABA levels in the baseline condition were stable over time in both groups (stress: $P = 0.41$; chocolate: $P = 0.13$; Fig. 2*C*) and no significant differences were found in DRN GABA basal concentrations between the two groups ($t = 1.19$; $df = 14$; $P = 0.253$, *SI Appendix*, Fig. S4*A*).

Consistent with a decrease in presynaptic GABA release, we found that exposure to both acute stress and white chocolate reduced the frequency, but not the amplitude, of mIPSCs recorded from 5-HT neurons with respect to the no stimulus group (frequency: $F(2, 44) = 11.75$, $P < 0.0001$; amplitude: $F(2, 44) = 1.45$, $P = 0.14$; Fig. 2*D*).

miR-34a Regulates DRN GABA Activity in Response to an Aversive, but Not a Rewarding Experience. MiR-34a has been shown to modulate neurotransmitter release (23, 24, 27–29). We thus tested whether it regulates GABA release in response to stimuli with different valence. Region-specific blockade of miR-34a was obtained by intra-DRN injection of an antagomiR-34a (Ant-34a) in C57BL/6 mice. A significant reduction of miR-34a levels in the DRN was detected after 2 h, 48 h, and 7 d from the injection with Ant-34a (10 µM, 0.5 µL), but not with a nontargeting scrambled sequence (negative control) (*SI Appendix*, Fig. S5). The rapid and long-lasting effect of this antagomiR is consistent with previous reports (27, 32).

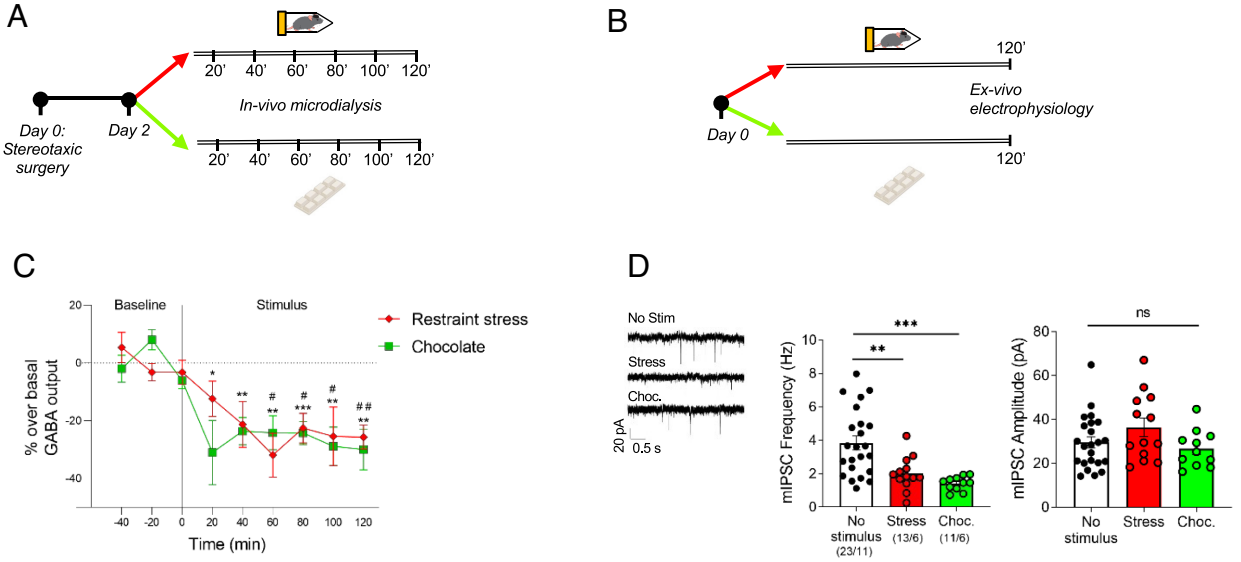

Fig. 2. Exposure to aversive (restraint stress) or rewarding (white chocolate) stimuli causes a local reduction of GABA release in the DRN. (*A* and *B*) Experimental timelines: GABA release was evaluated by: (A) in vivo microdialysis during, or (B) ex vivo electrophysiology after, 120 minutes of exposure to restraint stress or white chocolate. (*C*) In vivo microdialysis shows that exposure to 120 min of restraint stress or white chocolate promotes a significant reduction of GABA levels in the DRN of C57BL/6 mice (restraint group N = 8; white chocolate group N = 8), $^{*}P < 0.05$, $^{**}P < 0.01$ from basal values (white chocolate group). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ from basal values (restraint stress group). Results are expressed as percent changes (mean ± SEM) from basal values (*D*) *Left* panel: example traces of voltage-clamp spontaneous recordings (in DNQX+TTX) from DRN 5-HT neurons of mice that had received either no stimulus, 2 h of restraint stress, or 2 h of chocolate exposure. *Right* panels: frequency but not amplitude of miniature inhibitory post-synaptic currents (mIPSCs, mean ± SEM) were significantly lower in mice exposed to stress or chocolate vs no stimulus ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns: non-significant). Number of cells per number of animals (n/N) are indicated below each group in panel relating to mIPSC frequency.

We then evaluated the effect of miR-34a blockade on the reduction of GABA release induced by restraint stress or chocolate exposure (Fig. 3 *A* and *B*). Of note, all animals exposed to chocolate showed similar latency to approach the palatable food [F(3, 42) = 1.006, $P = 0.474$] and no differences in the quantity of chocolate consumed [F(3, 42) = 2.055, $P = 0.141$], regardless of the experimental condition (*SI Appendix*, Fig. S3).

Regarding the stress experiments with microdialysis, we first checked that in baseline conditions, DRN GABA outflow was stable over time (Neg. Ctrl-assigned: $P = 0.30$, Ant-34a-assigned: $P = 0.62$; Fig. 3*C*) and that basal GABA concentrations were not different between the Ant-34a and the negative control groups (t = 1.233; df = 13; $P = 0.232$, *SI Appendix*, Fig. S4*B*). Then, mice were infused with Ant-34a or its negative control in the DRN, in concomitance with the stressful procedure (Fig. 3*A*): in these conditions, the decrease of GABA outflow induced by restraint stress was evident only in the negative control-injected group, and not in the Ant-34a injected one, indicating that GABAergic miR-34a is involved in the response to acute stress [main effect of treatment (F(1, 13) = 172.7; $P < 0.0001$)], with significant differences between the Ant-34a and negative control groups at 20, 40, 60, and 100 min (Fig. 3*C*).

Remarkably, intra-DRN injection of Ant-34a (performed 48 h before, Fig. 3*B*) did not modify local GABA release in mice exposed to chocolate when compared to negative control subjects. A significant reduction of GABA levels in the DRN was in fact observed in mice when exposed to chocolate regardless of the treatment [time factor: F(1, 13) = 4.511; $P = 0.0044$]. More specifically, in Ant-34a-treated mice, GABA levels were significantly lower at 20, 40, 60, 80, and 100 min (Fig. 3*D*), while in negative control-treated mice, significant differences from baseline were found at 20, 40, 100, and 120 min from the beginning of the experience. As in the previous microdialysis experiments, GABA release was stable over time in the baseline condition (Neg. Ctrl.: p = 0.88; Choc. Ant-34a: $P = 0.86$; Fig. 3*D*) and basal concentrations were not different between Ant-34a and negative control-injected mice (t = 1.114; df = 13; $P = 0.285$, *SI Appendix*, Fig. S4*C*).

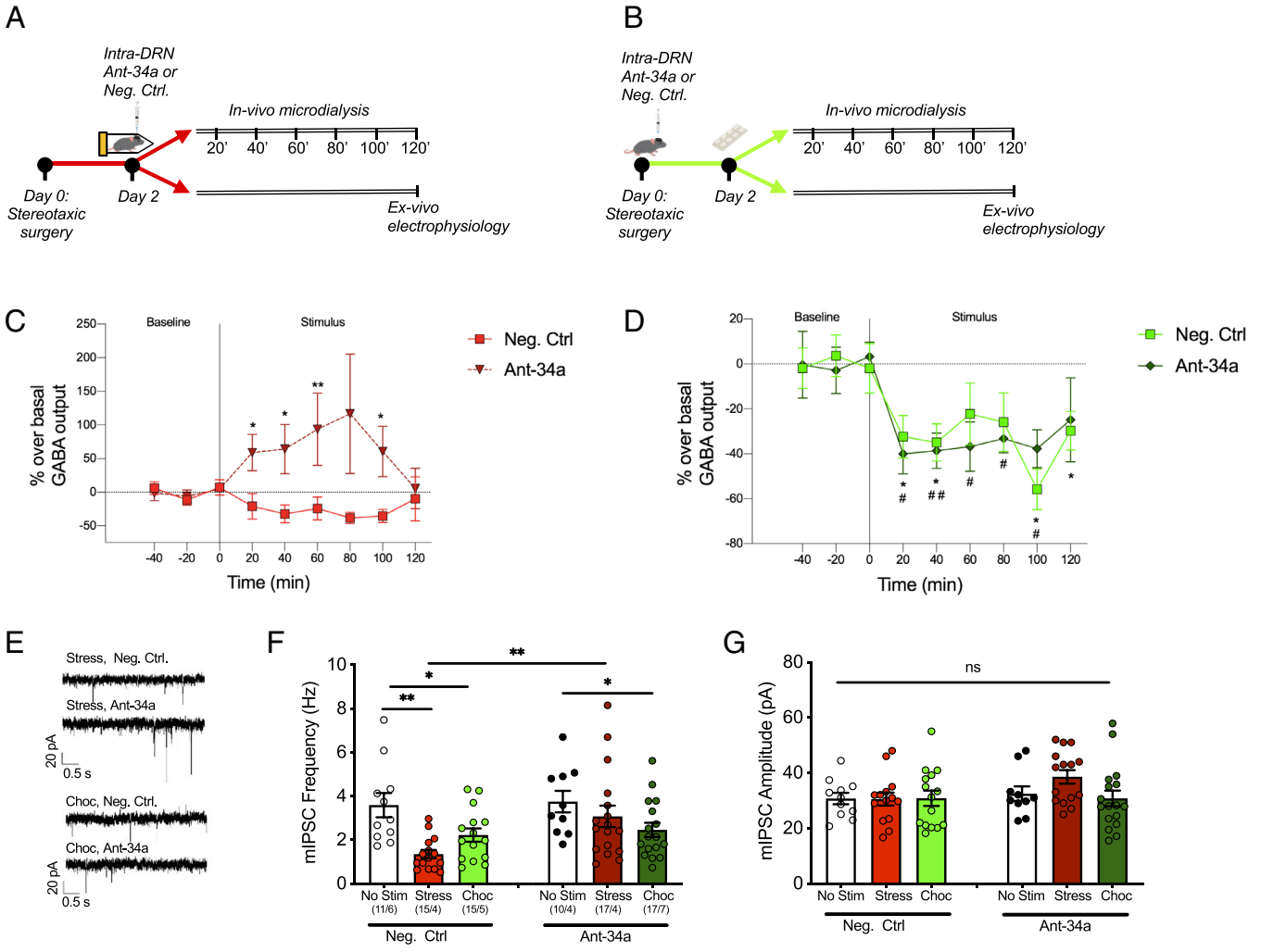

Fig. 3. Inhibition of miR-34a in the DRN through an antagomiR-34a (Ant-34a) potentiates local GABA release in response to an aversive (restraint stress), but not a rewarding (white chocolate), stimulus. Experimental timelines: (*A*) Two days after the stereotaxic surgery, Ant-34a, or negative control (Neg. Ctrl.) were administered. Immediately after the administration, the 2 h restraint stress procedure started. (*B*) In case of the rewarding stimulus protocol, Ant-34a or Neg. Ctrl. was administered 2 d before exposure to white chocolate, to avoid immobilization-induced stress. (*C*) In vivo microdialysis data show that intra-DRN infusion of Ant-34a in C57BL/6 mice exposed to 120 min of restraint stress (N = 7) caused a time-dependent increase of GABA outflow compared to the Neg. Ctrl.-treated group (N = 8), $^{*}P < 0.05$ in comparison with the corresponding time point of the Neg. Ctrl. group. (*D*) Conversely, 120 min of white chocolate exposure significantly reduced GABA outflow regardless of the Ant-34a/Neg.Ctrl. treatment (Ant-34a group N = 6; Neg.Ctrl. Group N = 9), $^{*}P < 0.05$, from basal values (Neg. Ctrl. -treated group). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ from basal values (Ant-34a-treated group). Microdialysis results are expressed as percent changes (means ± SEM) from basal values. (*E*) Representative traces of mIPSCs. (*F* and *G*) mean ± SEM of mIPSC frequency and amplitude recorded from 5-HT neurons in the absence of any stimulus, at the end of the restraint protocol or of the exposure to chocolate, in animals infused with Ant-34a or Neg. Ctrl., $^{*}P < 0.05$, $^{**}P < 0.01$, ns: non-significant. Number of cells per number of animals (n/N) are indicated below each group in panel *F*.

GABAergic transmission evaluated through electrophysiological experiments performed at the end of the aversive or rewarding experience revealed a similar pattern (Fig. 3 *E*–*G*): a two-way ANOVA analysis showed a significant effect of stimulus [F(2,79) = 6.858, $P = 0.002$] and treatment [F(1,79) = 4.539, $P = 0.036$; interaction: F(2,79) = 2.542, $P = 0.08$] on mIPSC frequency and no significant effects on mIPSC amplitude. Further *post hoc* analysis revealed that exposure to chocolate reduced mIPSC frequency in both the Ant-34a and the negative control groups, while restraint stress elicited a decrease of frequency only in the negative control group and not in the Ant-34a one.

Similar results were obtained when Ant-34a was infused 48 h before the restraint stress exposure (*SI Appendix*, Fig. S6): A two-way ANOVA analysis on mIPSC frequency showed a significant interaction between stimulus and treatment [F(1,79) = 10.07. $P = 0.002$], while no significant effects were found on mIPSC amplitude. *Post hoc* comparison confirmed that exposure to restraint stress reduced mIPSC frequency only in the negative control group and not in the Ant-34a one.

These findings indicate that miR-34a expressed within the DRN selectively controls local GABA release and inhibitory constraint over 5-HT neurons in an adverse situation. Since we identified DRN miR-34a exclusively in GABAergic neurons, we tested whether these responses to stress were selectively controlled by miR-34a-expressing GABAergic neurons. To this end, we measured GABA release in response to restraint in the DRN of Gad2::Cre/miR-34$^{loxP/loxP}$ and in the two control groups: Gad2::Cre and miR-34$^{loxP/loxP}$ mice (timeline in *SI Appendix*, Fig. S7*A*). In in vivo microdialysis experiments, GABA levels in baseline were stable over time (Gad2::Cre: $P = 0.89$, miR-34$^{loxP/loxP}$ mice: $P = 0.16$, Gad2::Cre/miR-34$^{loxP/loxP}$: $P = 0.34$; *SI Appendix*, Fig. S7*B*) and no significant difference in basal GABA concentrations was found among the three groups of mice [F(2, 24)=0.177; $P = 0.838$, *SI Appendix*, Fig. S4*D*]. In response to restraint stress, we found a significant genotype x time interaction [F(2, 24) = 2.279; $P = 0.011$; *SI Appendix*, Fig. S7*B*]. Specifically, while Gad2::Cre and miR-34$^{loxP/loxP}$ showed a slight, non-significant, decrease of GABA release in the DRN, Gad2::Cre/miR-34$^{loxP/loxP}$ mice showed a significant increase of GABA release at all time points measured. *Post hoc* comparison with Gad2::Cre/miR-34$^{loxP/loxP}$ mice revealed significant differences at 20 to 120 min compared to Gad2::Cre as well as to miR-34$^{loxP/loxP}$ mice. Consistently, electrophysiology recordings from 5-HT neurons showed a significant effect of genotype on mIPSC frequency [F(2, 66) = 3.84; $P = 0.026$; *SI Appendix*, Fig. S7*C*). *Post hoc* analysis showed that restraint stress caused a significant increase in Gad2::Cre/miR-34$^{loxP/loxP}$ mice compared to miR-34$^{loxP/loxP}$ ($P = 0.018$) and to Gad2::Cre mice ($P = 0.037$). No significant differences were evident for mIPSC amplitude [F(2, 66) = 0.77, $P = 0.47$; *SI Appendix*, Fig. S7*C*].

Finally, we carried out a further control to test the intra-DRN origin of miR-34a^{+}-GABAergic neurons involved in controlling GABA release on 5-HT neurons in stress conditions. Thus, we blocked miR-34a expression in the DRN via local injection of an adeno-associated virus (AAV) expressing CRE-recombinase (or control AAV) in miR-34$^{loxP/loxP}$ mice and measured GABAergic transmission in DRN 5-HT neurons. Electrophysiology recordings showed a significantly higher mIPSCs frequency in stressed mice that had received intra-DRN injection of AAV-Cre compared to control (AAV-Scra)-injected mice (t = 2.119, df = 24, $P = 0.045$). The mIPSCs amplitude was not different between the two groups (*SI Appendix*, Fig. S8). These results further support the intra-DRN origin of miR-34a^{+}-GABAergic neurons involved in the response to stress.

GABAergic miR-34a in the DRN Is Necessary for the Behavioral Response to Aversive, but Not Rewarding Stimuli. In rewarding experiences, inhibition of DRN GABAergic neurons facilitates both active (approach/consumption) and passive (waiting) responses (2). On the contrary, in aversive contexts, it is associated with passive behavior elicited by intense/severe threat situations (4).

In the experiments involving chocolate consumption that we described above, the latency to approach chocolate and the amount consumed indicated that inactivation of miR-34a in the DRN did not affect behavioral measures of reward (*SI Appendix*, Fig. S3). However, for the aversive contexts, the restraint apparatus does not allow to measure a behavioral response. Thus, we performed additional experiments to test whether inhibition of DRN miR-34a by local Ant-34a infusion or genetic deletion of miR-34a in GABAergic neurons influences behavior expressed in negative-valence contexts, such as the dark–light test (DLT) and the forced swim test (FST, Fig. 4 *A* and *E*). Moreover, we tested whether the same manipulations of miR-34a influenced sucrose preference scores (Fig. 4 *A* and *E*), a standard measure of a positive hedonic response.

Our results show that sucrose preference scores were insensitive to DRN Ant-34a infusion (t = 1.032; df = 15; $P = 0.318$, Fig. 4*B*) and to genetic deletion of miR-34a in GABAergic neurons [main effect of genotype: F(2, 23) = 0.2694, $P = 0.766$; Fig. 4*F*]. These data are in line with the lack of effects of DRN Ant-34a infusion on chocolate-induced approach and consumption responses and support the finding that miR-34a expressed in the DRN or by GABAergic neurons is not involved in the behavioral outcome of rewarding experiences.

When mice underwent an aversive experience, the effect of inhibiting miR-34a (by both local infusion of Ant-34a or by genetic inhibition) differed depending on the type of experience. Indeed, Ant-34a infusion in the DRN did not modify avoidance behavior in DLT, measured by the time spent (t = 0.212; df = 15; $P = 0.834$) and the number of entries (t = 0.399; df = 15; $P = 0.695$) in the light compartment (Fig. 4*C*). Moreover, avoidance expressed by Gad2::Cre/miR-34$^{loxP/loxP}$ mice in the DLT was not changed in comparison with that expressed by the two control groups (Fig. 4*G*). Indeed, a significant main effect of the factor genotype [F(2, 23) = 4.197, $P = 0.0279$) was due to Gad2::Cre mice spending significantly less time in the lit zone than miR-34$^{loxP/loxP}$ mice (Fig. 4*G*, *Left*). No significant differences were evident in the number of entries in the light compartment in the three groups [F(2, 23) = 3.128; $P = 0.062$; Fig. 4*G*, *Right*). By contrast, DRN Ant-34a infusion (t = 4.511; df = 15; $P = 0.0004$; Fig. 4*D*) or genetic deletion of miR-34a in GABAergic neurons reduced immobility expressed in the FST [main effect of genotype (F(2, 23) = 12.18; $P = 0.0002$; Fig. 4*H*)].

Discussion

The DRN plays a fundamental role in orchestrating behavioral responses to motivationally salient stimuli, regardless of their positive or negative valence, through 5-HT neurons projecting toward many brain areas (1, 6, 11, 12). Although a consistent population of GABAergic neurons modulates the activity of 5-HT neurons within the DRN, no study has identified and characterized DRN GABAergic neurons engaged in responses to motivationally salient stimuli. Our study starts to fill this void by identifying the selective expression of miR-34a in a subpopulation of GABAergic neurons and offering evidence of some of their functional characteristics.

In this work, we present several innovative findings: 1) miR-34a is expressed in GABAergic neurons of the DRVL (Dorsal Raphè

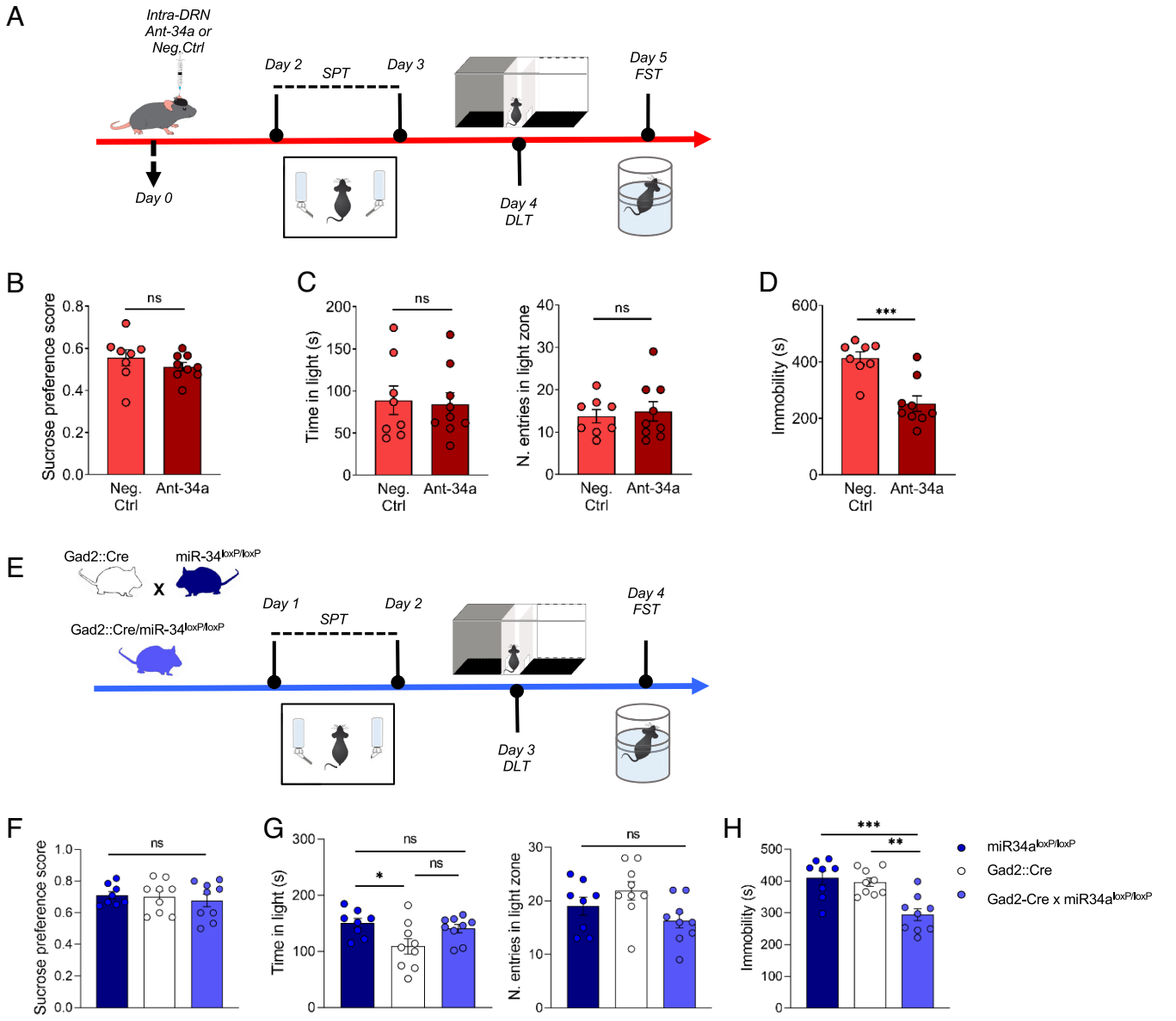

Fig. 4. Inhibition of miR-34a in the DRN reduces immobility in the FST, while leaving the behavior in the DLT and the SPT unaltered. (*A*) Experimental timeline: antagomiR-34a (Ant-34a) or negative control (Neg. Ctrl) were administered 2 d before the start of the behavioral tests. (*B–D*) Intra-DRN infusion of Ant-34a did not alter sucrose consumption in the SPT (panel *B*) nor the time spent and the number of entries in the light compartment of the DLT (panel *C*), while it significantly reduced the immobility time in the FST (panel *D*) compared to Neg. Ctrl-treated mice. (*E*) Experimental timeline: behavioral characterization of mice with selective inhibition of miR-34a in GABAergic neurons. (*F–H*) The behavioral profile of mice carrying a genetic deletion of miR-34a specifically in GABAergic neurons (Gad2::Cre/miR-34$^{loxP/loxP}$ mice) was compared to their relative control groups (Gad2::Cre and miR-34$^{loxP/loxP}$ mice). (*F*) No significant differences were observed in the SPT among the 3 groups. (*G*) In the DLT, the time spent and the number of entries in the light zone of Gad2::Cre/miR-34$^{loxP/loxP}$ mice was not significantly different compared to miR-34$^{loxP/loxP}$ and to Gad2::Cre mice. (*H*) In the FST, Gad2::Cre/miR-34a$^{loxP/loxP}$ mice showed a significant decrease of immobility time compared to Gad2::Cre and miR-34$^{loxP/loxP}$ mice. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ns: nonsignificant.

Ventrolateral Nucleus); 2) miR-34a modulates DRN GABAergic transmission in a valence-dependent manner; 3) miR-34a modulation of GABAergic transmission in the DRN selectively mediates the behavioral response to severe adverse experiences.

DRN neurons receive GABAergic inputs from both local interneurons and distal GABAergic cells originating in other brain structures (13). Our combined miRNAscope and ISH approach showed that the miR-34a signal is localized in soma-shaped structures within the DRVL, a small region highly responsive to negative emotional stimuli (17, 33–35). Moreover, this technique, by allowing analysis of the miR-34a and of the GAD67 signals on the same slice, demonstrated that miR-34a is expressed in DRVL GABAergic neurons. The cell-type specificity of miR-34a expression was further confirmed by ISH on a mouse model carrying a genetic deletion of miR-34a on GABAergic neurons. This finding is in line with another study on miRNA profiles in the mouse brain, showing that the miR-34 family and more specifically the a and c isoforms are expressed in GABAergic neurons of the cerebellum (21).

Our data on DRN inhibition of miR-34a with Ant-34a and AAV-Cre together with its expression pattern in the DRVL strongly suggest that local miR-34a-expressing GABAergic neurons are the ones regulating the response to stress. Yet, future experiments directly manipulating DRN miR-34a-positive GABAergic neurons possibly with an optogenetic approach will be required to definitively test this hypothesis.

Notably, we found that not all GABAergic neurons in the DRN colocalize with the miR-34a signal, but whether neurons expressing miR-34a correspond to a specific subtype of GABAergic cells remains to be determined. Indeed, in the DRN, inhibitory interneurons are morphologically, electrically, and chemically heterogeneous (13, 36), and a recent single-cell transcriptome study in mice reported at least three GABAergic neuronal subtypes in this area (8). Future studies will clarify whether miR-34a is expressed in a specific subpopulation of DRN GABAergic cells.

The findings reported here support the relevance of DRN GABAergic transmission in responding to emotionally salient stimuli regardless of their valence (2, 4). Further, they identify a specific role of locally expressed miR-34a in regulating GABAergic transmission in the DRN under an acute aversive experience but not during exposure to palatable food. The modulation of GABAergic transmission by miR-34a is in line with previous evidence reporting a role of this same miRNA in acute regulation of synaptic transmission (27). Moreover, many mRNAs encoding proteins that modulate neurotransmitter release in the DRN are validated targets of miR-34a, including 5-HT2C (22), CRFR1 (37, 38), or synaptotagmin-1 (29).

In the experiments involving chocolate exposure, all mice showed approaching and consummatory behavior, indicating that this stimulus represents a positive experience. This behavior could depend on either the caloric and/or the palatable aspect of the stimulus. Indeed, restricted feeding as well as restricted caloric intake have been reported to alter the behavioral and neural responses to this natural reward (31, 39). Yet, in our experiments, mice were subjected to a standard feeding regime, arguing in favor of the rewarding nature of the stimulus.

In response to a rewarding stimulus, both active (approach/consumption) and passive (waiting) responses are associated with inhibition of DRN GABAergic neurons (2). On the other hand, in adverse experiences, active (escape or avoid) and passive (immobility) behavioral responses are associated with increased and decreased GABA activity, respectively (4). In line with the latter observation, we report a decrease in GABA release in the DRN of mice exposed to palatable food or to a severe and inescapable stressor such as the restraint stress. However, only in mice exposed to the stressor, inhibition of DRN GABA release was dependent on miR-34a. These results suggest a leading role of miR-34a in regulating coping behavior during severe aversive conditions, and the behavioral data in our study support the proposed model. The FST is both a severe and an inescapable experience (40). On the other hand, the aversive experience that characterizes the DLT can be easily avoided by not entering the lit compartment. Interestingly, both pharmacological inhibition of DRN miR-34a and genetic deletion of GABAergic miR-34a did not affect avoidance of the aversive compartment of the DLT. On the contrary, these manipulations effectively reduced the expression of the passive defensive response (immobility) in the FST-exposed mice.

The reduction of escaping attempts is considered an adaptive coping response to an aversive inescapable situation. Indeed, it prevents a useless loss of energies, thus allowing to exploit unpredictable opportunities to come out of the situation (41, 42). Therefore, the reduction of GABAergic transmission mediated by miR-34a in the DRN could represent a step in the successful adaptation to a severe aversive experience. The conceptual framework of the Research Domain Criteria (RDoC) guidelines (43–45) provides another appealing interpretation. According to the RDoC, behavioral responses to real/acute and to potential threats are considered two different constructs of human behavior within the negative valence domain and therefore could be driven by different neural circuits. As proposed by Söderlund and Lindskog (45), the DLT (or similar tests) evaluates the behavioral response to a potential threat, while the FST measures the behavioral response to a real (acute) one.

Finally, the observation that different measures of reward-elicited behavior (chocolate consumption and sucrose preference) were unaffected by our miR-34a manipulations supports the conclusion that miR-34a identifies a circuit within the DRN selectively engaged in the behavioral response to specific environmental challenges.

When activated, the DRN provides 5-HT to distinct brain regions and this activity is finely controlled by local GABAergic circuits (13–15). Thus, stimuli with different valence could activate distinct subpopulations of GABAergic cells. These would in turn impinge on different 5-HT neurons targeting distinct areas, such as the ventral tegmental area for reinforcing effects (1, 46) or the mPFC for behavioral responses to specific aversive conditions (6, 14, 24). In line with this hypothesis, discrete subsets of GABAergic cells encoding valence-specific information were recently reported in the mPFC and central amygdala (47, 48). Our data indicate that DRN miR-34a contributes to constrain GABAergic release on a subset of local 5-HT neurons, likely facilitating 5-HT release in selected target structures. Supporting this idea, we have previously reported that both constitutive and conditional deletion of miR-34a in mice attenuates severe stress-induced 5-HT release in the mPFC (23, 24, 28). These considerations point to miR-34a expressing GABAergic neurons in the DRVL as a possible node of a DRN-mPFC circuit mediating the behavioral response to severe negative conditions.

In conclusion, this work expands our knowledge of miRNAs regulatory activity in the brain, suggesting that they can represent a functional signature of distinct neuronal (sub)populations with valence-specific activity in the brain. Moreover, the identification of the biological underpinning of specific valence system behaviors is crucial to the development of new treatments based on the pathophysiology of psychiatric disease, moving a step further toward precision medicine for mental disorders.

Materials and Methods

For details on *Materials and Methods*, see *SI Appendix*.

Animals. Twelve- to fifteen-week-old male mice were used for experiments. All experiments were conducted in accordance with the Italian national law on the European Community Council Directives (2010/63/UE). Gad2::Cre/miR-34$^{loxP/loxP}$ mice were obtained by crossing Gad2::Cre with miR-34$^{loxP/loxP}$ (*SI Appendix*, Fig. S9).

Aversive or Rewarding Stimuli. Restraint stress and white chocolate were used as aversive and rewarding stimulus, respectively (24, 49).

AntagomiR-34a Injection. For the restraint stress condition, antagomiR-34a (Ant-34a) or its negative control (both 0.5 μL, 10 μM, Qiagen) was administered at 0.25 μL/min flow rate either right after cannula implantation or immediately before the stressful stimulus initiation. For experiments involving reward stimulus-induced GABA evaluation, as well as for behavioral tests, Ant-34a, or its negative control, was infused 48 h before the stimulus/test, to avoid the confounding effect of immobilization-induced stress. The following coordinates from the bregma were used: AP = + 4.36 mm, ML = + 1.5 mm, DV = + 2.0 mm, angled 26°.

In Vivo Microdialysis. A microdialysis probe of 3.5 mm length was implanted with the following coordinates: AP = + 4.36 mm, ML = + 1.5 mm, angled 26°. GABA concentrations were detected using a high-performance liquid chromatography system coupled with a fluorescence detector.

Ex Vivo Electrophysiological Recordings. Electrophysiological recordings were performed from acute coronal brain sections containing the DRN. Whole-cell patch-clamp recordings were obtained from putative 5-HT neurons, identified

based on their location, morphology, and electrophysiological characteristics (50–52), as explained in detail in the methods section of the *SI Appendix* and in *SI Appendix*, Fig. S10. mIPSCs were recorded in voltage clamp mode at −70 mV in the presence of DNQX and TTX. For *post hoc* identification, neurons were filled with Alexa Fluor 488 through the recording pipette.

ISH. ISH on DRN coronal slices was performed as described previously (24).

miRNAscope™. miRNAscope™ (Advanced Cell Diagnostic, USA) was performed according to the manufacturer's protocol as in ref. 53. Before miRNAscope processing (probe SR-mmu-miR-34a-5p-S1 for miR-34a), sections were incubated with primary antibodies (anti-GAD67, anti-5-HT). Sections were examined under a confocal laser-scanning microscope.

Behavioral Tests. Tests were run from the least to the most invasive, and a minimum interval of 24 h between tests was maintained, to decrease the chance that behavioral responses would be altered by prior test history.

Sucrose preference test (SPT). During familiarization (day 1) and the day of the test (day 2), animals were individually placed in the test cage and exposed to a double choice drinking test [saccharin solution (1%) or drinking water]. Results are expressed as sucrose preference score [sucrose intake/(sucrose intake+water intake)].

DLT. The DLT apparatus consisted in a rectangular box divided by a partition into 2 environments: a dark (35×20×30 cm) and a brightly illuminated compartment (35×20×30 cm). The compartments were connected by a small passage located at the bottom of the partition, in the center. The time spent (s) and the number of entries in the aversive, lighted, compartment within a 5-minute recording, were annotated.

FST. Mice were individually placed in a glass cylinder (diameter 18 cm, height 40 cm) containing 20 cm of fresh tap water maintained at 28 ± 2 °C during a single session lasting 10 min. The behavior of the animals was recorded and then scored by a trained observer blind to the animals' genotype and treatment.

Statistics. Data are presented as mean ± SEM. Datasets were analyzed with *t* test or ANOVA, based on the experimental design (see *SI Appendix* for more details).

Data, Materials, and Software Availability. The datasets generated and analyzed in the current study are available at: 10.5281/zenodo.7947677 (54).

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1. H. L. Wang *et al.*, Dorsal Raphe dual serotonin-glutamate neurons drive reward by establishing excitatory synapses on VTA mesoaccumbens dopamine neurons. *Cell Rep.* **26**, 1128–1142.e7 (2019).
2. Y. Li *et al.*, Serotonin neurons in the Dorsal Raphe nucleus encode reward signals. *Nat. Commun.* **7**, 10503 (2016).
3. M. Luo, J. Zhou, Z. Liu, Reward processing by the Dorsal Raphe nucleus: 5-HT and beyond. *Learn. Mem.* **22**, 452–460 (2015).
4. C. Seo *et al.*, Intense threat switches Dorsal Raphe serotonin neurons to a paradoxical operational mode. *Science* **363**, 539–542 (2019).
5. A. Sengupta, A. Holmes, A discrete Dorsal Raphe to basal amygdala 5-HT circuit calibrates aversive memory. *Neuron* **103**, 489–505.e7 (2019).
6. J. Ren *et al.*, Anatomically defined and functionally distinct Dorsal Raphe serotonin sub-systems. *Cell* **175**, 472–487.e20 (2018).
7. J. Y. Cohen, M. W. Amoroso, N. Uchida, Serotonergic neurons signal reward and punishment on multiple timescales. *Elife* **4**, e06346 (2015).
8. K. W. Huang *et al.*, Molecular and anatomical organization of the Dorsal Raphe nucleus. *Elife* **8**, e46464 (2019).
9. J. Ren *et al.*, Single-cell transcriptomes and whole-brain projections of serotonin neurons in the mouse dorsal and median raphe nuclei. *Elife* **8**, e49424 (2019).
10. B. W. Okaty *et al.*, A single-cell transcriptomic and anatomic atlas of mouse Dorsal Raphe Pet1 neurons. *Elife* **9**, 1–44 (2020).
11. G. E. Paquelet *et al.*, Single-cell activity and network properties of Dorsal Raphe nucleus serotonin neurons during emotionally salient behaviors. *Neuron* **110**, 2664–2679.e8 (2022).
12. J. Baruni, L. Luo, Illuminating complexity in serotonin neurons of the Dorsal Raphe nucleus. *Neuron* **110**, 2519–2521 (2022).
13. F. Hernández-Vázquez, J. Garduño, S. Hernández-López, GABAergic modulation of serotonergic neurons in the Dorsal Raphe nucleus. *Rev. Neurosci.* **30**, 289–303 (2019).
14. M. Waselus, R. J. Valentino, E. J. Van Bockstaele, Collateralized Dorsal Raphe nucleus projections: A mechanism for the integration of diverse functions during stress. *J. Chem. Neuroanat.* **41**, 266–280 (2011).
15. L. Boothman, J. Raley, F. Denk, E. Hirani, T. Sharp, In vivo evidence that 5-HT(2C) receptors inhibit 5-HT neuronal activity via a GABAergic mechanism. *Br. J. Pharmacol.* **149**, 861–869 (2006).
16. L. Huang *et al.*, A retinoraphe projection regulates serotonergic activity and looming-evoked defensive behaviour. *Nat. Commun.* **8**, 14908 (2017).
17. K. Spoida, O. A. Masseck, E. S. Deneris, S. Herlitze, Gq/5-HT2c receptor signals activate a local GABAergic inhibitory feedback circuit to modulate serotonergic firing and anxiety in mice. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 6479–6484 (2014).
18. K. S. Kosik, The neuronal microRNA system. *Nat. Rev. Neurosci.* **7**, 911–920 (2006).
19. G. Schratt, MicroRNAs at the synapse. *Nat. Rev. Neurosci.* **10**, 842–849 (2009).
20. E. McNeill, D. Van Vactor, MicroRNAs shape the neuronal landscape. *Neuron* **75**, 363–379 (2012).
21. M. He *et al.*, Cell-type-based analysis of microRNA profiles in the mouse brain. *Neuron* **73**, 35–48 (2012).
22. L. Lo Iacono *et al.*, MicroRNA-34a regulates 5-HT2C expression in Dorsal Raphe and contributes to the anti-depressant-like effect of fluoxetine. *Neuropharmacology* **190**, 108559 (2021).
23. L. Lo Iacono *et al.*, MicroRNA-34a regulates the depression-like behavior in mice by modulating the expression of target genes in the Dorsal Raphè. *Mol. Neurobiol.* **57**, 823–836 (2020).
24. D. Andolina *et al.*, MicroRNA-34 contributes to the stress-related behavior and affects 5-HT prefrontal/GABA amygdalar system through regulation of corticotropin-releasing factor receptor 1. *Mol. Neurobiol.* **55**, 7401–7412 (2018).
25. O. Issler *et al.*, MicroRNA 135 is essential for chronic stress resiliency, antidepressant efficacy, and intact serotonergic activity. *Neuron* **83**, 344–360 (2014).
26. A. Baudry, S. Mouillet-Richard, B. Schneider, J. M. Launay, O. Kellermann, miR-16 targets the serotonin transporter: A new facet for adaptive responses to antidepressants. *Science* **329**, 1537–1541 (2010).
27. B. Berentsen *et al.*, MicroRNA-34a acutely regulates synaptic efficacy in the adult dentate gyrus in vivo. *Mol. Neurobiol.* **57**, 1432–1445 (2020).
28. D. Andolina *et al.*, Effects of lack of microRNA-34 on the neural circuitry underlying the stress response and anxiety. *Neuropharmacology* **107**, 305–316 (2016).
29. M. Agostini *et al.*, Neuronal differentiation by TAp73 is mediated by microRNA-34a regulation of synaptic protein targets. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 21093–21098 (2011).
30. E. M. McNeill *et al.*, The conserved microRNA miR-34 regulates synaptogenesis via coordination of distinct mechanisms in presynaptic and postsynaptic cells. *Nat. Commun.* **11**, 1092 (2020).
31. E. C. Latagliata, S. Puglisi-Allegra, R. Ventura, S. Cabib, Norepinephrine in the medial pre-frontal cortex supports accumbens shell responses to a novel palatable food in food-restricted mice only. *Front. Behav. Neurosci.* **12**, 7 (2018).
32. E. M. Jimenez-Mateos *et al.*, Silencing microRNA-134 produces neuroprotective and prolonged seizure-suppressive effects. *Nat. Med.* **18**, 1087 (2012).
33. M. Matthiesen, A. Spiacci, H. Zangrossi, Effects of chemical stimulation of the lateral wings of the Dorsal Raphe nucleus on panic-like defensive behaviors and Fos protein expression in rats. *Behav. Brain Res.* **326**, 103–111 (2017).
34. A. J. Jasinska, C. A. Lowry, M. Burmeister, Serotonin transporter gene, stress and raphe-raphe interactions: A molecular mechanism of depression. *Trends Neurosci.* **35**, 395–402 (2012).
35. L. T. K. Crawford, C. P. Craige, S. G. Beck, Increased intrinsic excitability of lateral wing serotonin neurons of the Dorsal Raphe: A mechanism for selective activation in stress circuits. *J. Neurophysiol.* **103**, 2652–2663 (2010).
36. L. H. Calizo *et al.*, Raphe serotonin neurons are not homogenous: Electrophysiological, morphological and neurochemical evidence. *Neuropharmacology* **61**, 524–543 (2011).
37. S. Haramati *et al.*, MicroRNA as repressors of stress-induced anxiety: The case of amygdalar miR-34. *J. Neurosci.* **31**, 14191–14203 (2011).
38. B. G. Dias *et al.*, Amygdala-dependent fear memory consolidation via miR-34a and Notch signaling. *Neuron* **83**, 906–918 (2014).
39. E. Patrono *et al.*, When chocolate seeking becomes compulsion: Gene-environment interplay. *PLoS One* **10**, e0120191 (2015).
40. K. G. Commons, A. B. Cholanians, J. A. Babb, D. G. Ehlinger, The rodent forced swim test measures stress-coping strategy, not depression-like behavior. *ACS Chem. Neurosci.* **8**, 955–960 (2017).
41. S. Cabib, S. Puglisi-Allegra, The mesoaccumbens dopamine in coping with stress. *Neurosci. Biobehav. Rev.* **36**, 79–89 (2012).
42. E. R. De Kloet, M. L. Molendijk, Coping with the forced swim stressor: Towards understanding an adaptive mechanism. *Neural Plast.* **2016**, 6503162 (2016).
43. T. R. Insel, The NIMH research domain criteria (RDoC) project: Precision medicine for psychiatry. *Am. J. Psychiatry* **171**, 395–397 (2014).
44. E. Anderzhanova, T. Kirmeier, C. T. Wotjak, Animal models in psychiatric research: The RDoC system as a new framework for endophenotype-oriented translational neuroscience. *Neurobiol. Stress* **7**, 47–56 (2017).
45. J. Söderlund, M. Lindskog, Relevance of rodent models of depression in clinical practice: Can we overcome the obstacles in translational neuropsychiatry? *Int. J. Neuropsychopharmacol.* **21**, 668–676 (2018).

46. C. J. Browne *et al.*, Dorsal Raphe serotonin neurons inhibit operant responding for reward via inputs to the ventral tegmental area but not the nucleus accumbens: Evidence from studies combining optogenetic stimulation and serotonin reuptake inhibition. *Neuropsychopharmacology* **44**, 793–804 (2019).
47. K. A. Cummings, S. Bayshtok, T. N. Dong, P. J. Kenny, R. L. Clem, Control of fear by discrete prefrontal GABAergic populations encoding valence-specific information. *Neuron* **110**, 3036–3052.e5 (2022).
48. T. Yang *et al.*, Plastic and stimulus-specific coding of salient events in the central amygdala. *Nature* **616**, 510–519 (2023).
49. R. Ventura, E. C. Latagliata, C. Morrone, I. La Mela, S. Puglisi-Allegra, Prefrontal norepinephrine determines attribution of "high" motivational salience. *PLoS One* **3**, e3044 (2008).
50. Y. Jin *et al.*, Sodium salicylate suppresses GABAergic inhibitory activity in neurons of rodent Dorsal Raphe nucleus. *PLoS One* **10**, e0126956 (2015).
51. S. Marinelli *et al.*, Serotonergic and nonserotonergic Dorsal Raphe neurons are pharmacologically and electrophysiologically heterogeneous. *J. Neurophysiol.* **92**, 3532–3537 (2004).
52. L. G. Kirby, L. Pernar, R. J. Valentino, S. G. Beck, Distinguishing characteristics of serotonin and non-serotonin-containing cells in the Dorsal Raphe nucleus: Electrophysiological and immunohistochemical studies. *Neuroscience* **116**, 669–683 (2003).
53. F. Wang *et al.*, RNAscope: A novel in situ RNA analysis platform for formalin-fixed, paraffin-embedded tissues. *J. Mol. Diagn.* **14**, 22 (2012).
54. D. Andolina, C. Marchetti, Datasets for "GABAergic miR-34a regulates Dorsal Raphè inhibitory transmission in response to aversive, but not rewarding, stimuli". *Zenodo*. https://doi.org/10.5281/zenodo.7947677. Deposited 18 May 2023.