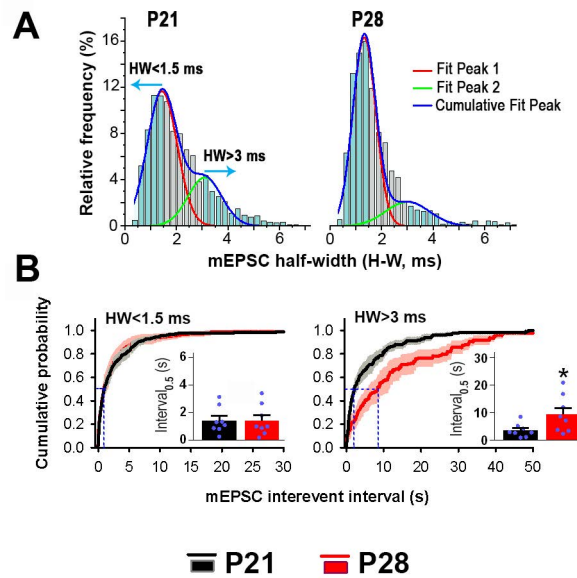


**Fig. S1. Postnatal modifications in the size of glutamate and GABA**

**synaptic afferents to the DRN.** Violin plots illustrating global sizes (in voxels) of synaptic boutons identified with array tomography during postnatal life. **(A)** VGLUT1 (Kruskal-Wallis statistic = -505.2,  $p < 0.0001$ ): \* $p < 0.0001$  for P4 vs. P21, P28 and P60; \* $p < 0.0001$  for P7 vs. P21, P28 and P60; \* $p < 0.0001$  for P14 vs. P21, P28 and P60; \* $p < 0.0001$  for P60 vs. P21 and P28. P4 ( $n=1363$ ), P7 ( $n=870$ ), P14 ( $n=1722$ ), P21 ( $n=2760$ ), P28 ( $n=2760$ ) and P60 ( $n=1795$ ). **(B)** VGLUT2 (Kruskal-Wallis statistic = -289.5,  $p < 0.0001$ ): \* $p < 0.0001$  for P4 vs. P14, P21, P28 and P60; \* $p < 0.0001$  for P7 vs. P14, P21, P28 and P60; \* $p < 0.0006$  for P14 vs. P28 and P60; \* $p < 0.0001$  for P21 vs. P28 and P60; \* $p < 0.006$  for P28 vs. P60. P4 ( $n=2049$ ), P7 ( $n=1079$ ), P14 ( $n=3132$ ), P21 ( $n=4321$ ), P28 ( $n=2036$ ) and P60 ( $n=3964$ ). **(C)** GAD2 (Kruskal-Wallis statistic = -864.0,  $p < 0.0001$ ): \* $p < 0.006$  for P4 vs. P21, P28 and P60; \* $p < 0.0001$  for P7 vs. P21, P28 and P60; \* $p = 0.0126$  for P14 vs. P21 and \* $p < 0.0001$  for P14 vs. P28 and P60; \* $p < 0.0001$  for P21 vs. P28 and P60, and for P28 vs. P60. P4 ( $n=1600$ ), P7 ( $n=925$ ), P14 ( $n=2990$ ), P21 ( $n=3239$ ), P28 ( $n=3353$ ) and P60 ( $n=2717$ ). Multiple comparisons were done by Dunn's test. Medians and quartiles are shown.



**Fig. S2. Kinetic analysis of mEPSCs at P21 and P28.**

(A) To further study kinetic properties of mEPSCs, half-width (HW) durations were defined as the time between the rising and decay phases of each mEPSC at 50% of the peak amplitude. Histograms of mEPSC HW duration were fitted to a double-peak Gaussian distribution at both P21 and P28 ( $n=8$  neurons from 3 mice/age):

$$y = y_0 + \left( A_1/w_1 * \sqrt{\pi/2} \right) * \exp(-2 * ((x - x_{c1})/w_1)^2) + \left( A_2/w_2 * \sqrt{\pi/2} \right) * \exp(-2 * ((x - x_{c2})/w_2)^2),$$

where  $y_0$ ,  $x_c$ ,  $w$  and  $A$  represent offset, center, width, and area, respectively. For P21:  $y_0 = 1.04 \exp - 4 \pm 0.00$ ,  $x_{c1} = 1.43 \pm 0.02$ ,  $A_1 = 0.17 \pm 0.01$ ,  $w_1 = 1.35 \pm 0.05$ ,  $x_{c2} = 3.09 \pm 0.00$ ,  $A_2 = 0.07 \pm 0.01$ ,  $w_2 = 1.55 \pm 0.00$ ,  $R^2 = 0.96$ . For P28:  $y_0 = -3.42 \exp - 5 \pm 0.00$ ,  $x_{c1} = 1.32 \pm 0.02$ ,  $A_1 = 0.18 \pm 0.01$ ,  $w_1 = 1.05 \pm 0.05$ ,  $x_{c2} = 3.00 \pm 0.01$ ,  $A_2 = 0.04 \pm 0.00$ ,  $w_2 = 2.00 \pm 0.00$ .  $R^2 = 0.94$ .

Akaike's Information Criterion test (AIC) was used to compare single vs. double-peak

Gaussian models. For P21, a double-peak Gaussian model was  $8.69 \times 10^{15}$  times more likely to be correct (AIC = -527.55, Akaike weight = 1) than a single-peak Gaussian model (AIC = -454.14, Akaike weight =  $1.15 \times 10^{-16}$ ). For P28, a double-peak Gaussian model was 119588 times more likely to be correct (AIC = -401.94, Akaike weight = 0.99) than a single-peak Gaussian model (AIC = -378.45, Akaike weight =  $8.36 \times 10^{-6}$ ). **(B)** mEPSCs were sorted in two distinct groups according to their H-W duration: i) mEPSCs that had a HW duration shorter than 1.5 ms ( $\sim x_{C_1}$ ) and ii) mEPSCs that had a HW duration longer than 3 ms ( $\sim x_{C_2}$ ). The relative frequency of both groups is shown in light blue in the histogram **(A)**. Cumulative probability distribution of the inter-event interval for each group of mEPSCs was calculated at P21 and P28 (n=8 neurons from 3 mice/age). The mean inter-event interval at a cumulative probability of 0.5 was calculated using a single exponential function  $y = y_0 + a * \exp(-b * t)$ , where  $y_0$ ,  $a$ ,  $b$  and  $t$  represent offset, pre-exponential coefficient, time constant and time, respectively) (Interval<sub>0.5</sub>, blue dashed lines, inset). \*P<0.05, T-test with Welch's correction, t=2.303, df=8.832 (HW >3ms P21 vs. P28). P=0.9957, T-test, t=0.005, df=14 (HW <1.5ms P21 vs. P28).

**Table S1. Analysis of bursts in mIPSCs.**

	<b>P21</b>	<b>P28</b>	
<b>Events in burst</b>	30.0 ± 8.4 (7)	19.1 ± 1.9 (5)	Mann-Whitney U = 13.0, p = 0.5
<b>Burst duration (s)</b>	3.1 ± 0.4 (7)	4.1 ± 1.4 (5)	Mann-Whitney U = 17.0, p = 0.1
<b>Mean intraburst interval (ms)</b>	151.0 ± 42.0 (7)	252.2 ± 95.1 (5)	Mann-Whitney U = 10.0, p = 0.3
<b>Frequency (Hz)</b>	34.3 ± 5.1 (7)	18.8 ± 3.8 (5)*	T-test $t_{10} = 2.3$ , p = 0.046
<b>Mean intraburst amplitude (-pA)</b>	18.9 ± 3.8 (7)	23.8 ± 5.1 (5)	T-test $t_{10} = 1.1$ , p = 0.3
<b>Mean amplitude outside the burst (-pA)</b>	16.7 ± 1.7 (7)	21.6 ± 4.4 (5)	Mann-Whitney U = 10.0, p = 0.3

Values are expressed as mean±s.e.m. (n). There were no differences between the mean amplitude inside vs. outside of the burst for both P21 and P28. In P21:  $t_{12} = 0.494$ , p = 0.630; In P28: Mann-Whitney U = 11, p = 0.841.