Supplemental informations

Chauveau *et al*. Stress modulation by NPS

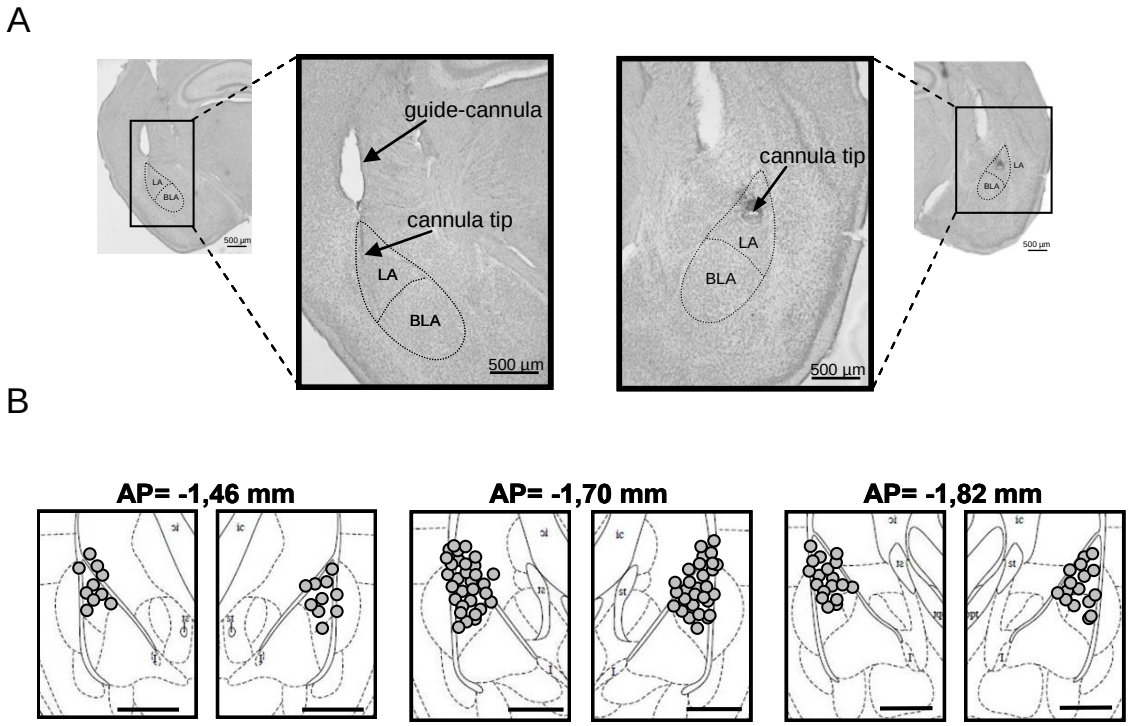

Figure S1: Histological controls for lateral amygdala injections. **(A)** Pictures of a representative histological control from brain of two different animals 1day (left) or 10 days after injection (right). All animal showing the cannula tip and/or infusion site into the lateral amygdala (LA) were included in the behavioral analysis. Animals showing injection site outside the LA were discarded from the analysis. **(B)** Schematic drawings of coronal sections show the location of the needle injector tip of representative implanted animals (grey dots= injection site). BLA: basolateral amygdaloid complex; LA: lateral amygdala. The anterio-posterior (AP) coordinate (from bregma) is mentioned for each diagram. The drawings were adapted Paxinos G & Franklin KBJ (2001) The Mouse Brain in Stereotaxic Coordinates. 2nd ed.

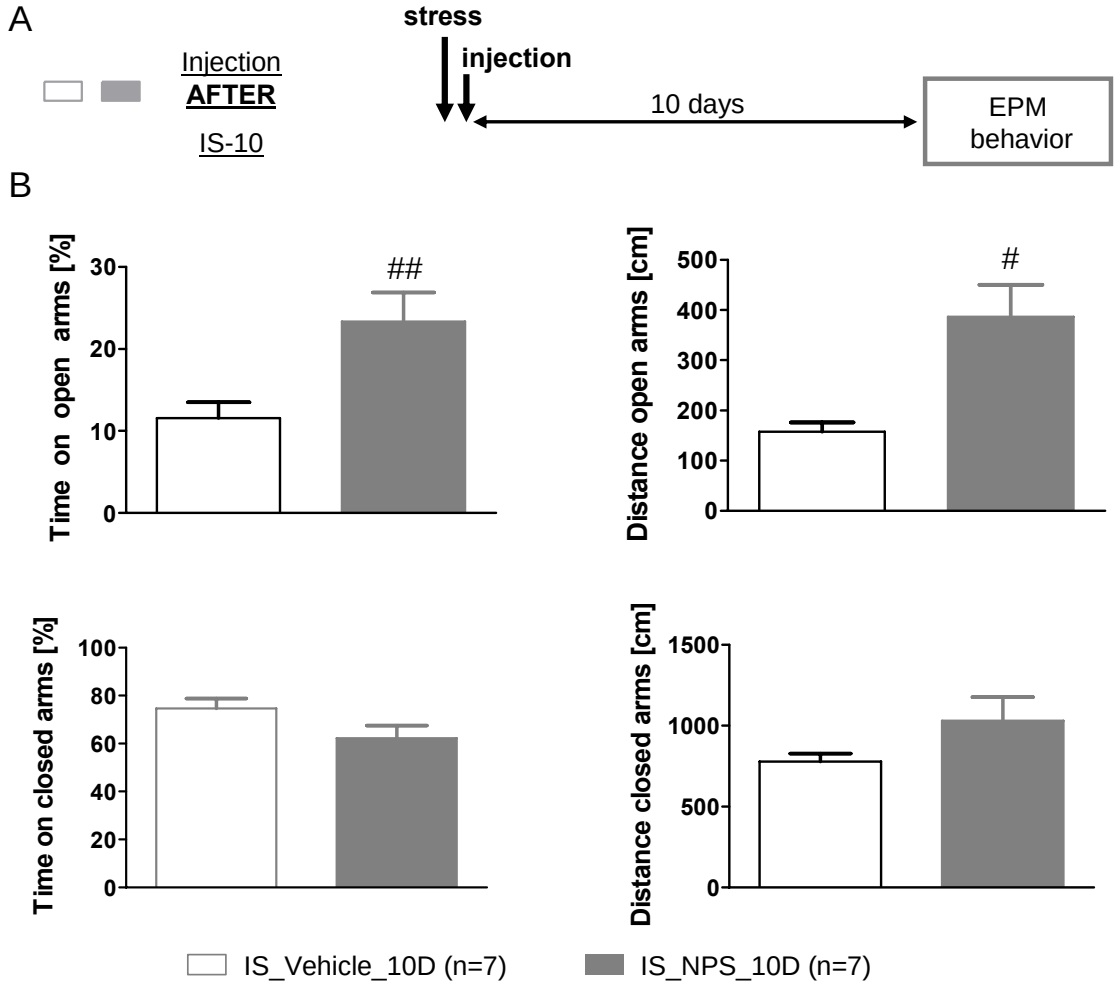

Figure S2: Effect of NPS injection into the LA after IS-10D on elevated-plus maze behavior. (A) Experimental design. Injection of vehicle (IS_Vehicle_10D) or NPS (IS_NPS_10D) immediately (<1 min) after IS, and EPM test 10 days later. **(B)** Elevated-Plus-Maze behavioral results. Drug effect (Student t-test): ##: $p<0.01$, #: $p<0.05$.

Animals receiving NPS injection immediately after stress showed an increase of distance and time in open arms in the EPM, similar to that observed with NPS injection 20 min before IS. No differences were found for the time and the distance in closed arms

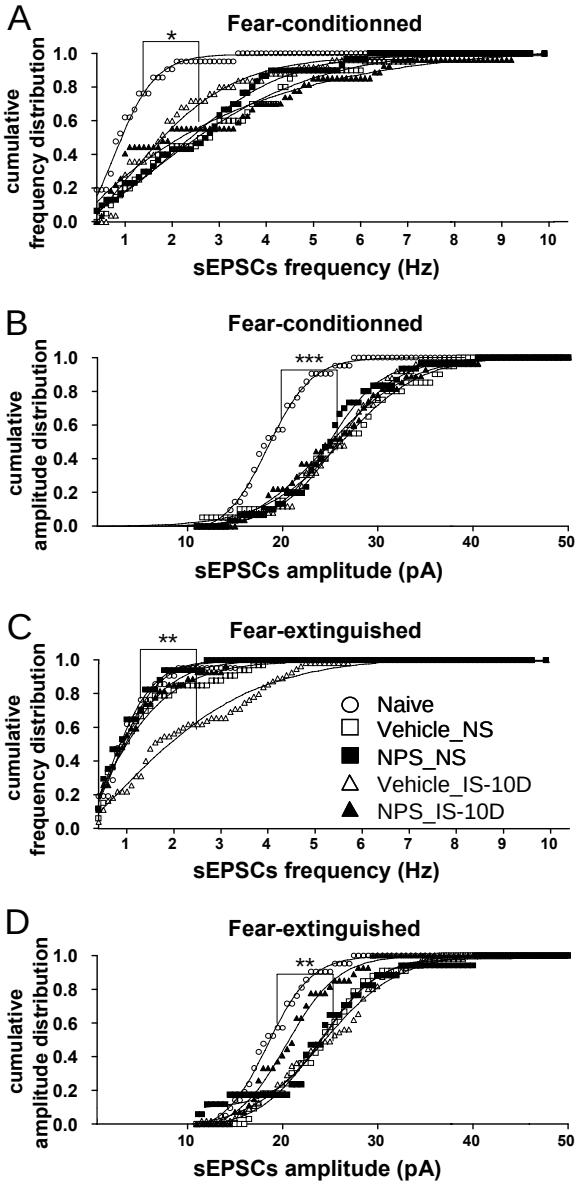

Figure S3: Cumulative frequency distribution of sEPSCs in LA projection neurons after fear conditioning (A) and fear extinction (C). **Cumulative amplitude distribution** after fear conditioning (B) and fear extinction (D). **Excitatory synaptic activity in the LA: influence of conditioned fear, stress and NPS.** Recordings from the same cells as in Fig. 4. Comparison to non-trained controls: ***: p<0.001, **: p<0.01, *: p<0.05.

Fear conditioning induced a right shift of the cumulative frequency distribution as compared to naïve animals, indicating the appearance of higher frequencies induced by fear conditioning (two-sample Kolmogorov-Smirnov test (K-S)-test: $p<0.05$). This right shift was independent

of vehicle or NPS-injection and stress exposure. In line with these findings, the cumulative amplitude distribution of the analyzed sEPSCs was increased as compared to sEPSC amplitudes retrieved from naïve animals ($p<0.001$; K-S-test). After fear extinction, only frequencies of the recorded sEPSCs of stressed, vehicle-injected animals showed a significant right shift in the cumulative distribution plot as compared to naïve animals ($p<0.01$; K-S-test), indicating the appearance of increased frequencies in stress-exposed, vehicle-injected animals. The cumulative amplitude distribution of the recorded sEPSCs indicates that only stressed, NPS-injected animals show a not significantly different amplitude distribution as compared to naïve animals, whereas the sEPSC amplitudes retrieved from the other groups show a shift to significantly elevated amplitudes compared to the naïve control group ($p<0.01$; K-S-test).

Behavioral group		Number of cells / animals	Membrane resting potential (mV)	Resting input resistance (MΩ)	Instant spike frequency (Hz)
non-trained	Veh / NPS NS	40 4	-82.5 ± 1.1	509 ± 38	12.9 ± 1.0
fear conditioned	Vehicle NS	20 2	-80.0 ± 1.3	409 ± 36$^{\#}$	14.4 ± 1.3
	NPS NS	30 3	-81.5 ± 0.7	398 ± 27$^{\#\#}$	14.6 ± 1.3
	Vehicle IS-10D	25 3	-83.2 ± 1.1	416 ± 35$^{\#}$	15.7 ± 1.4
	NPS IS-10D	26 3	-84.7 ± 0.7	359 ± 17$^{\#\#\#}$	13.4 ± 0.7
fear extinguished	Vehicle NS	33 4	-75.3 ± 1.7$^{\#\#\#}$	375 ± 20$^{\#\#\#}$	12.9 ± 1.1
	NPS NS	17 2	-74.8 ± 1.7$^{\#\#\#}$	325 ± 23$^{\#\#\#}$	12.4 ± 1.6
	Vehicle IS-10D	55 6	-77.8 ± 1.2$^{\#\#}$	384 ± 21$^{\#\#\#}$	13.6 ± 0.7
	NPS IS-10D	27 3	-75.4 ± 1.6$^{\#\#\#}$	304 ± 32$^{\#\#\#}$	14.7 ± 2.0

Table S1: Intrinsic properties of LA projection neurons

NS: non-stressed animals, injection of NPS (NPS_NS) or vehicle (Vehicle_NS) bilaterally into the LA 10 days before fear conditioning. IS-10 D: exposure to IS 10 days before fear conditioning, with injection of NPS (NPS_IS-10D) or vehicle (Vehicle_IS-10D) 20 min before IS. Slices prepared 10 day after substance injection (non-trained), 24 hours after retrieval of fear memory (fear-conditioned) and 24 hours after retrieval of fear extinction (fear-extinguished). Comparison of behavioral groups to non-trained group (Student t-test):

###: $p<0.001$, ##: $p<0.01$, #: $p<0.05$

The differences in sEPSC properties observed between groups of animals might partly be explained by differences in intrinsic properties of LA projection neurons. To elucidate this

issue, active and passive membrane properties of these neurons were analyzed in the various groups. Significant differences (as compared to naïve mice) were evident from a drop in resting input resistance in all fear trained groups often accompanied by a faster first action potential in spike trains, and, a more positive resting membrane potential and action potential threshold after fear extinction. Of note, no difference in intrinsic properties was detected in LA projection neurons between the NPS- and vehicle-treated IS groups after fear extinction, which might have contributed to the specific changes in sEPSCs properties. In particular, there was no significant difference in resting membrane potential (-77.8 mV *versus* -75.4 mV; $p>0.05$), resting input resistance (383±21 MΩ *versus* 304±32 MΩ; $p>0.05$), instantaneous frequency of the first two action potentials in response to a depolarizing current injection (14±1 Hz versus 15±2 Hz; $p>0.05$).