SOCIETÀ ITALIANA DI FARMACOLOGIA

MODELLO PER INVIO RELAZIONE DI METÀ E FINE PERIODO

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TIPOLOGIA DI BORSA RICEVUTA: Borsa di ricerca SIF per soggiorno all'estero

TIPOLOGIA DI RELAZIONE : relazione di fine periodo

TITOLO DELLA RELAZIONE: Synaptic plasticity in Valproic acid model of autistic spectrum disorders

RELAZIONE:

Autistic Spectrum Disorder (ASD) is a heterogeneous set of neurodevelopmental disabilities. ASD is characterized by altered social interaction, compromised verbal and nonverbal communication, stereotyped and repetitive behaviours, often associated with comorbid features, such as social and generalized anxiety. Both genetic and environmental factors are involved in the etiology of ASD. Many of the ASD-associated genes are involved in brain development, cortical organization, synaptogenesis and neurotransmission. Among the environmental factors involved in the pathogenesis of ASD, it has been well documented that prenatal exposure to the antiepileptic drug valproic acid (VPA) is associated with increased risk of neurodevelopmental delay and autistic symptoms in the offspring. Indeed, when given during gestation, VPA not only increases the risk for various congenital malformations (8,10), but also induces core autistic symptoms in the offspring, i.e., impaired communication, reduced sociability and stereotyped behaviours.

The altered social behaviour displayed by VPA-exposed rats may be due to either a deficit in social reward processing or to a more general inability to properly understand and respond to social signals, which has been hypothesized to be the consequence of abnormal activity of the brain reward circuit in social contexts (1, 14). The most important brain areas involved in reward and reward-related learning are the ventral tegmental area (VTA), the nucleus accumbens (NAc), and the prefrontal cortex (PFC), that form mesocorticolimbic (MCL) pathway and which is involved in motivation and reward-related behavior. The NAc is a major target of the mesolimbic dopaminergic system and the overall impact of NAc output on behaviour depends on relative activity of D1- vs D2-expressing medium spiny neurons (MSNs), therefor given the important role of NAc dopamine in rewarding forms of social interaction such as social play (13), we addressed the role of the NAc in the social impairment displayed by VPA-exposed rats (500 mg/Kg) by performing behavioural, neurochemical electrophysiological experiments in this brain area. Along this line, previous studies in our laboratory have

shown that prenatal exposure to VPA causes selective deficits in the social domain in the rat offspring such as reduced play responsiveness, sociability and social discrimination compared with SAL-exposed animals, confirm here that adolescent rats prenatally exposed to VPA show decreased social reward-related behaviours. Moreover since the activation of D1 and D2 receptors in corticolimbic regions is also important for the expression of social behavior in rodents (4, 16, 13, 9), we hypothesized that the social deficit displayed by VPA-exposed rats may be due to changes in dopaminergic neurotransmission in corticolimbic brain areas. We found that, compared to control animals, adolescent rats prenatally exposed to VPA showed increased expression of D2 dopamine receptors in the NAc. Starting from this evidence, we found that NAc MSNs of VPA-exposed animals show a significant depolarization of the resting membrane potential and increased excitability indicating higher firing probability in conditions of normal synaptic excitation (3). These changes are likely caused by altered Kir current density, known to determine the hyperpolarized value of membrane potential in normal MSNs (~-85 mV) and to affect their AP discharge pattern (11). Collectively, these findings suggest an increase in intrinsic excitability of MSNs of VPA-exposed animals. Proper activity of direct and indirect pathways underlies proper motor learning as well as the acquisition of reward-related behaviours. Moreover, since changes in local and distant connectivity in the brain have been proposed as a possible cause of autistic behaviour (4), we investigate the synaptic transmission in VPA rats model and, in preliminary data, we found that VPA-exposed rats show changes in glutamatergic transmission. This suggests a perturbation of the excitatory/inhibitory in VPA-exposed rats. Moreover NAc is characterized by endocannabinoid (eCB)-mediated long term depression (LTD) originally discovered in the NAc/ventral striatum (15). The mesocorticolimbic eCB system modulates a vast array of synaptic functions (15) and plays a key role in brain development. eCB dysfunction is implicated as a major causal factor synaptopathies linked to the NAc (7, 12, 6) and it has been recently involved in ASD (20).

During my work in Dr. Manzonì OJ. laboratoty I evaluated:

(1) excitatory/inhibitory synaptic transmission;

(2) eCB-mediated long term depression;

(3) neurodevelopmental effects of VPA-prenatal exposure

MATERIALS AND METHODS

Animals

On gestational day 12.5, females Wistar rats received a single intraperitoneal injection of either VPA or saline (SAL) (500mg/Kg). The experiments were carried out on the male offspring during adolescence (PNDs 30–45) and adulthood (PNDs 60-90). One male pup per litter from different litters per treatment group was used in each experiment.

Slice preparation

Adolescent (P30-P45) and adult (P60-90) male rats were deeply anesthetized with isoflurane and sacrificed. The brain was sliced (300 µm) on the coronal plane in a sucrose-based solution at 4°C (in mm as follows: 87 NaCl, 75 sucrose, 25 glucose, 2.5 KCl, 4 $MgCl_2$, 0.5 $CaCl_2$, 23 $NaHCO_3$ and 1.25 NaH_2PO_4). Immediately after cutting, slices containing the NAc were stored for 1 h at 32°C in a low calcium ACSF that contained (in mm) as follows: 130 NaCl, 11 glucose, 2.5 KCl, 2.4 $MgCl_2$, 1.2 $CaCl_2$, 23 $NaHCO_3$, 1.2 NaH_2PO_4, and were equilibrated with 95% O_2 5% CO_2 and then at room temperature until the time of recording.

Whole-Cell Patch Clamp and Extracellular field Recordings in Acute Brain Slices

For whole-cell patch-clamp experiments, neurons were visualized using an upright microscope with infrared illumination. The intracellular solution was based on K+gluconate (in mM as follows: 145 K+ gluconate, 3 NaCl, 1 MgCl2, 1 EGTA, 0.3 CaCl2, 2 Na+ ATP, and 0.3 Na+GTP, 0.2 163 cAMP, buffered with 10 HEPES). The pH was adjusted to 7.2 and osmolarity to 290-300 mOsm. Electrode resistance was 4-6 MOhm. Current-voltage (I-V) curves were made by a series of hyperpolarizing to depolarizing current steps for evaluate MSN excitability. Excitatory/ Inhibitory post-synaptic currents were recorded in presence of GABAZINE (GABA-A antagonist) and NBQX and APV (AMPA-NMDA antagonists) respectively. While miniature events were recorded in presence of TTX, a voltage-dependent sodium channels blocker. Both spontaneous/miniature post-synaptic currents were recorded at -70 mV.

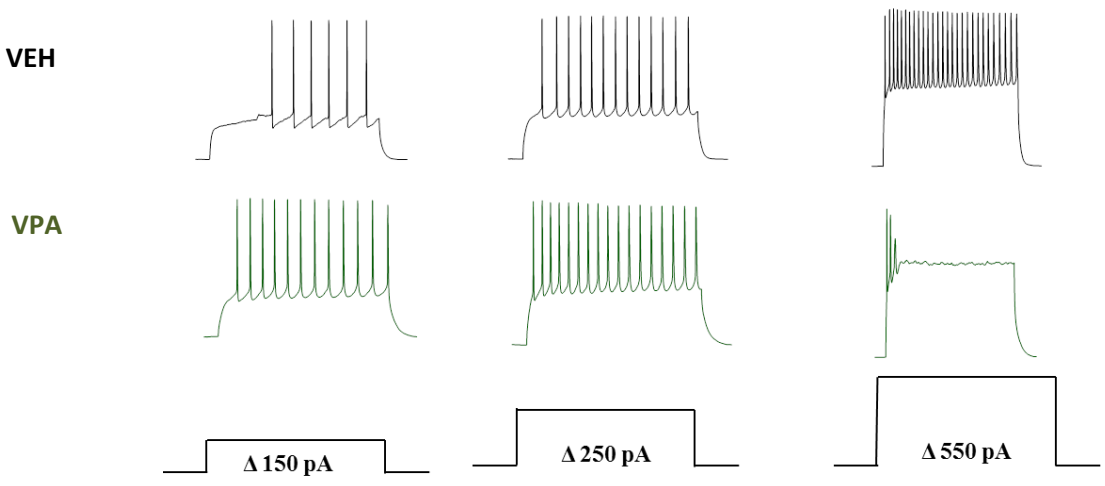
For extracellular field experiments, the recording pipette was filled with ACSF. The glutamatergic nature of the field EPSP (fEPSP) was systematically confirmed at the end of the experiments using the inotropic glutamate receptor antagonist CNQX (20 μM), which specifically blocked the synaptic component without altering the non-synaptic component.

RESULTS:

- **Effect of VPA-prenatal exposure on NAc-MSN Action Potential**

In Previous study we found that NAc-MSNs of VPA-exposed animals show a significant depolarization of the resting membrane potential and increased excitability, indicating higher firing probability in conditions of normal synaptic excitation (3). These changes are likely caused by altered Kir current density, known to determine the hyperpolarized value of membrane potential in normal MSNs (∼−85 mV) and to affect their AP discharge pattern (2,11). The large conductance, Ca2+-activated K+ channels (BKCa,KCa1.1) and sodium channels (NaV) are expressed in various brain neurons where they play important roles in regulating action potential duration, firing frequency and neurotransmitter release. Given their important role in the control of neuronal activity we decided to performed action potential analysis at three representative current steps (150, 250, 550). We found that, compared to control animals, adolescent rats prenatally exposed to VPA showed increases in action potential amplitude and significant reduction in afterhyperpolarization and inter events interval spikes (fig.1).

A



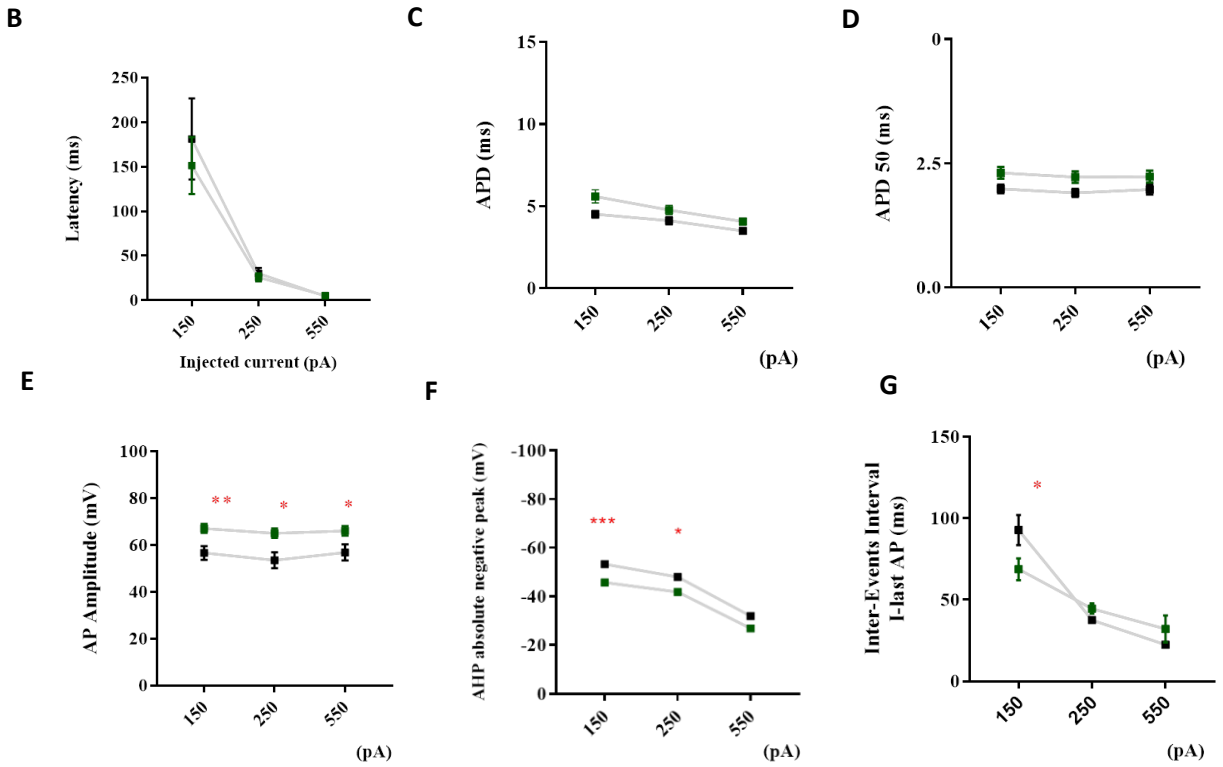
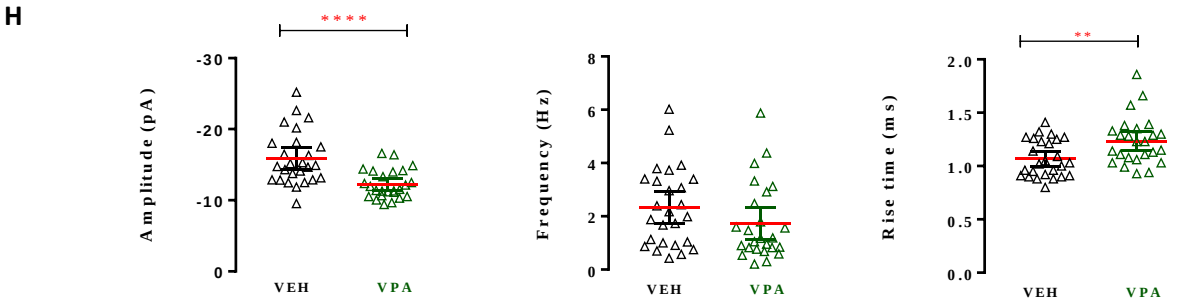


Fig. 1: Effect of Vpa-prenatal exposure on action potential: (A) Representatives voltage traces of VEH (black) and VPA (green) NAc-MSN evoked by depolarizing current steps. (B) Latency of first action potential, (C-D) action potential duration, (E) action potential amplitude, (F) afterhyperpolarization, (G) inter events interval. (VEH MSNs, n = 52; VPA MSNs, n = 59). Data represent mean values ± SEM; ∗p < 0.05, ∗∗p < 0.01, ∗∗∗p < 0.001 vs. VEH group (Student's t-test).

- **<u>Effect of VPA-prenatal exposure on NAc-MSN connectivity</u>**

Previous preliminary experiments have shown that VPA-prenatal exposure modified NAc-MSN synaptic transmission in VPA-exposed rats, based on these results I recorded both spontaneous (s)/miniature (m) excitatory(E) post-synaptic currents (s/mEPSCs) and inhibitory (I) post-synaptic currents (sIPSCs) in VEH and VPA exposed rats during adolescent (PND 30-45) (fig 2).



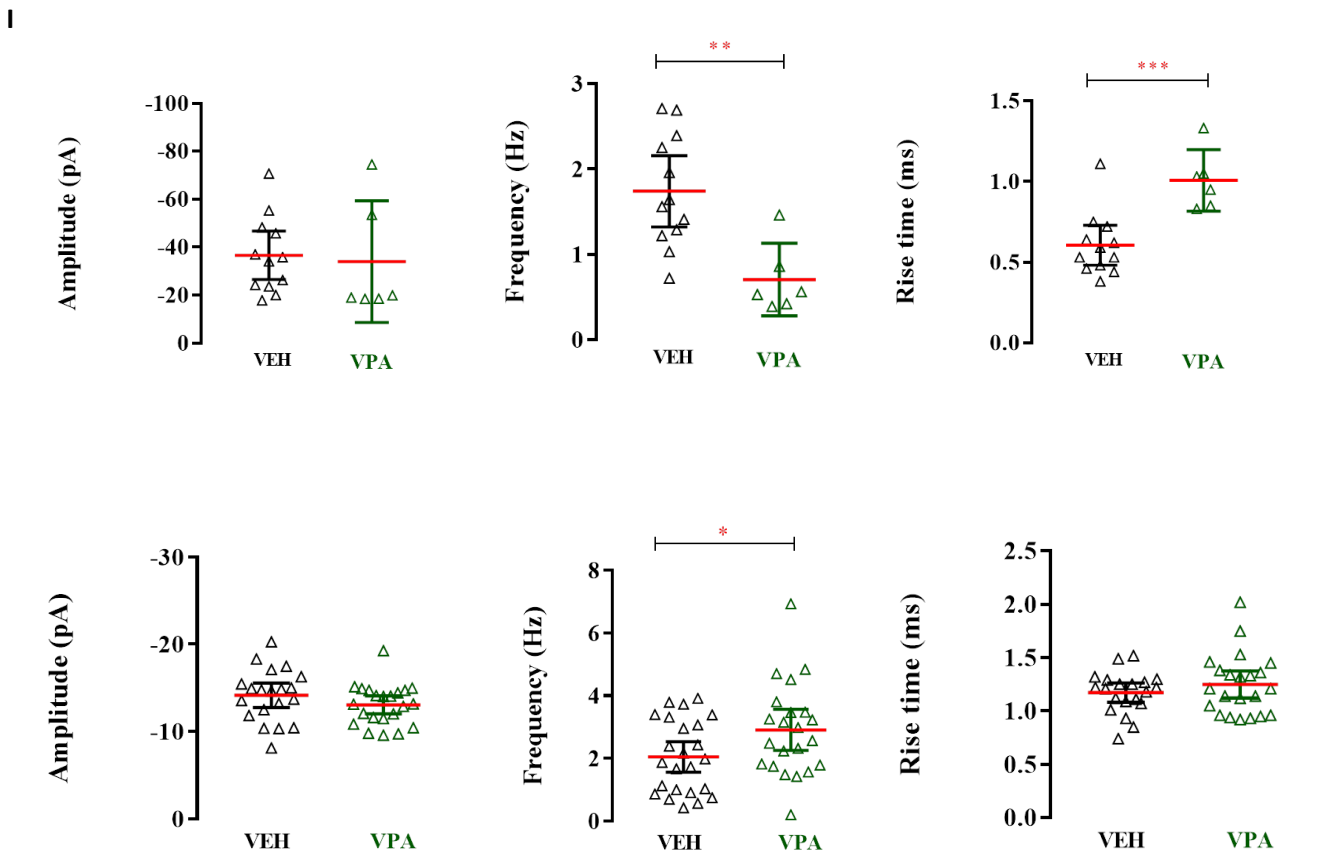


Fig. 2: Effect of Vpa-prenatal exposure on (H) sEPSCs (VEH MSNs, n = 26; VPA MSNs, n = 26), (I) sIPSCs (VEH MSNs, n = 12; VPA MSNs, n = 6) and (J) mEPSCs (VEH MSNs, n = 26; VPA MSNs, n = 26) amplitude, frequency and rise time. Data represent mean values ± SEM; ∗p < 0.05, ∗∗p < 0.01, ∗∗∗p < 0.001 vs. VEH group (Student's t-test).

We found that VPA-prenatal exposure significantly reduces both amplitude and rise time of sEPSCs without affect frequency on the contrary increases the frequency of mEPSCs in VPA-NAc-MSN compared to VEH group. Finally preliminary data on sIPSCs suggests a decreases in inhibitory NAc-MSN activity. These data can indicate alterations in connectivity and synaptic strength in VPA-exposed rats. Moreover we studied eCB-LTD in VEH rats in which low frequency stimulation protocol produces significant depression (fig.3). eCB-LTD was analyzed by comparing fEPSP amplitudes before and after 40 min after 10 Hz stimulation train. I used only VEH animals for eCB-LTD experiments because of COVID-19 emergency. I could not conclude these experimental groups with VPA-exposed rats since all researches have been suppressed starting from March the 16 and at the end of the emergency we haven't vpa adolescent rats.

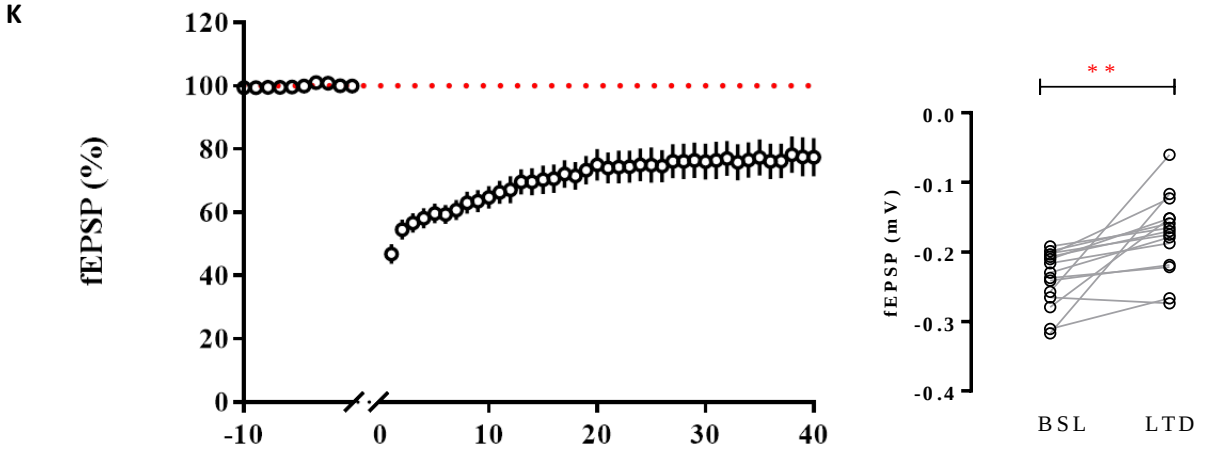
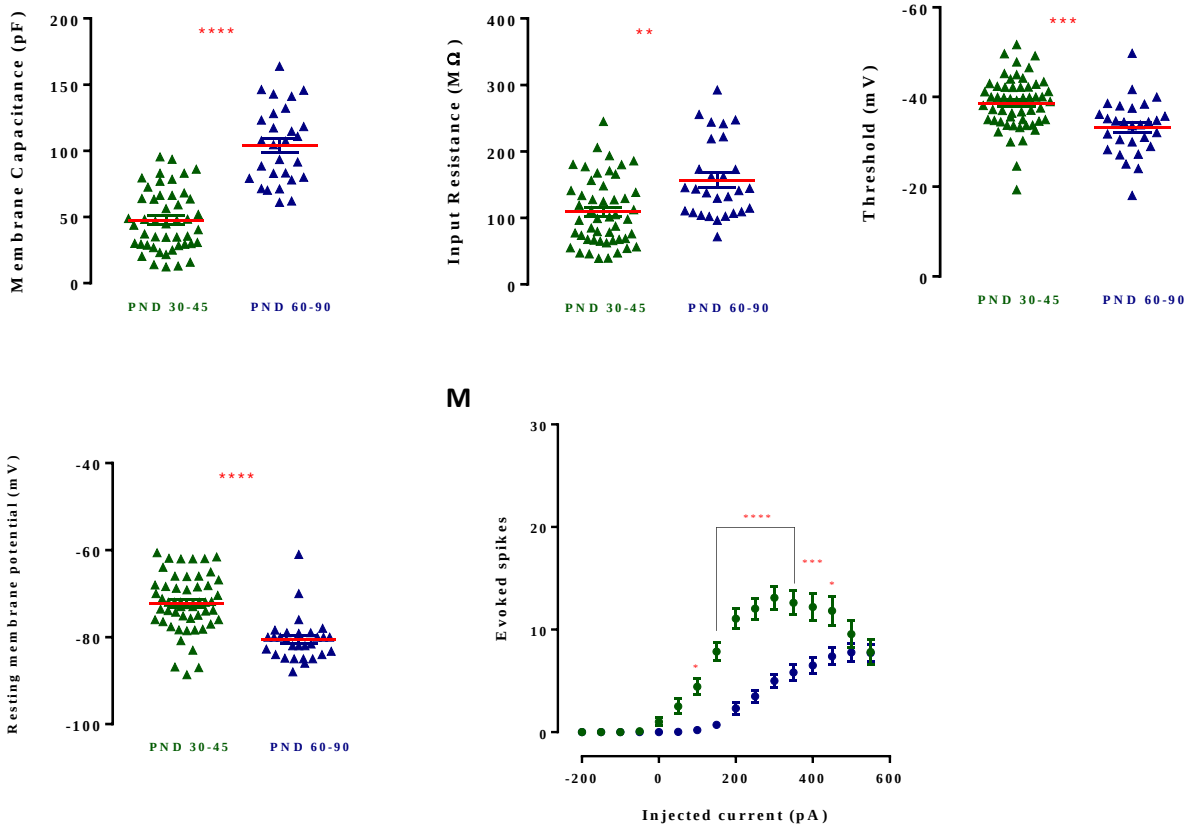


Fig. 3: (K) Baseline normalized fEPSP recordings from NAc after 10 Hz stimulation protocol in VEH rats. Averaged fEPSP amplitudes before (baseline) and 40 min after (LTD) 10 Hz stimulation protocol. Peak amplitude measurements before (baseline) and after (LTD) stimulation protocol from individual experiments (right). Data represent mean values ± SEM; ∗p < 0.05, ∗∗p < 0.01, ∗∗∗p < 0.001 vs. VEH group (Student's t-test).

- **Age-dependent effect of VPA-prenatal exposure on NAc-MSN**

To compared the post-natal effect of VPA during adolescent and adulthood we performed the same experiment in adult VPA rats (PND 60-90) to evaluate a possible aging link of VPA-prenatal exposure. We found significant different in passive membrane properties, intrinsic excitability and synaptic transmission between young and adult VPA-exposed rats (fig. 4-5).

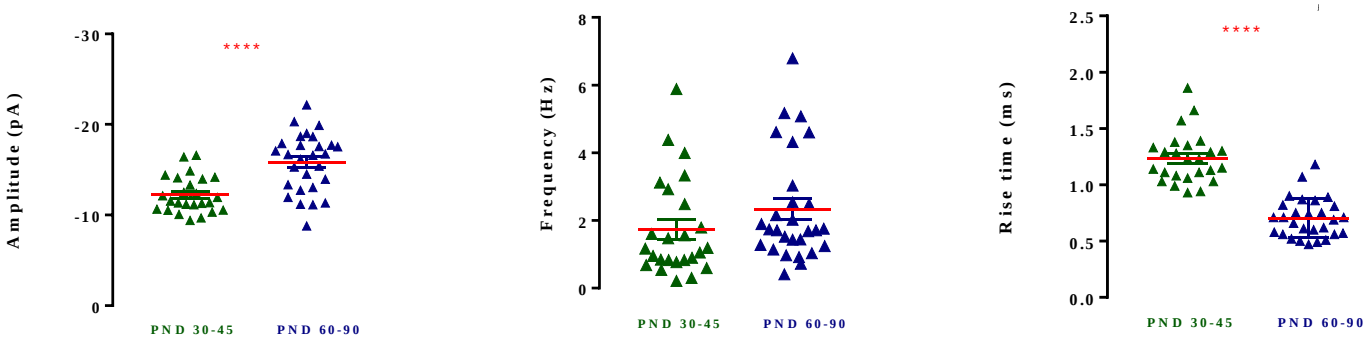
L



M

Fig. 4: (L) Passive membrane properties of adolescent (green) and adult (blue) VPA MSNs, membrane capacitance; input resistance; AP threshold; resting membrane potential. (M) Number of APs fired plotted against current steps of increasing value (VPA 30-45, n = 49; VPA 60-90, n = 28). Data represent mean values ± SEM; ∗p < 0.05, ∗∗p < 0.01, ∗∗∗p < 0.001 vs. VPA adolescents group (Student's t-test).

N



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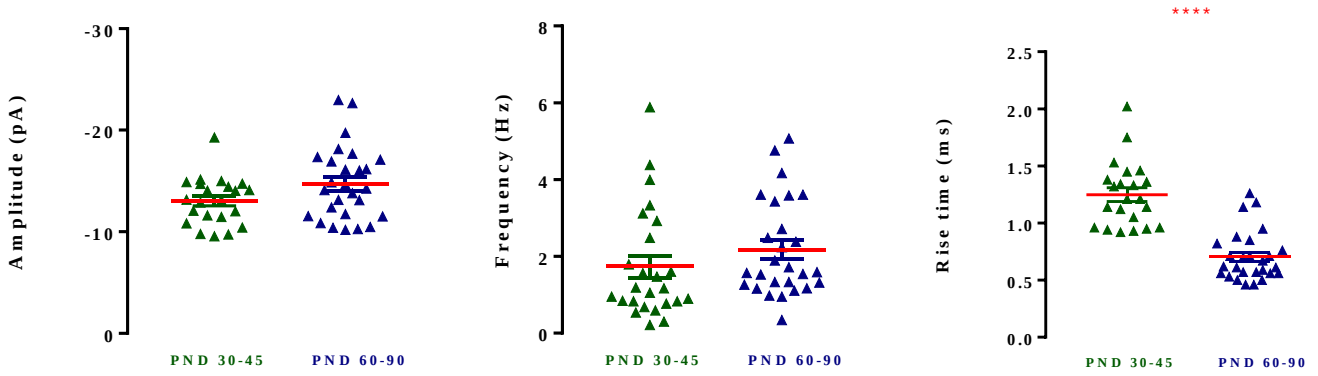

Fig. 5: Effect of VPA-prenatal exposure on (N) sEPSCs and (O) mEPSCs amplitude, frequency and rise time. (VPA 30-45, n = 25; VPA 60-90, n = 28). Data represent mean values ± SEM; ∗p < 0.05, ∗∗p < 0.01, ∗∗∗p < 0.001 vs. VPA adolescents group (Student's t-test).

- **Conclusion**

Clinical studies have repeatedly reported that maternal use of VPA during pregnancy can induce a wide range of abnormalities in the exposed children, ranging from structural malformations to more subtle autistic-like behaviors. For this reason, prenatal VPA exposure is nowadays considered an environmental risk factor involved in the pathogenesis of ASD. Based on the robust clinical evidence, prenatal exposure to VPA in rodents has been validated as a drug-induced preclinical model of ASD (17,18). As it has been hypothesized for autistic patients (1), the wide range of social dysfunctions displayed by VPA-exposed rats may be due to either their inability to properly percept, understand and respond to socially relevant cues, or to a failure of their brain reward system to assign a positive value to the social experience (15). Brain regions involved in the control of social behaviour include corticolimbic structures like NAc and their altered functionality may represent one neural substrate contributing to the social impairments characteristic of ASD (19, 1). In previous study, we found that prenatal exposure to VPA causes selective deficits in the social domain in the rat offspring at different developmental periods, together with a significant depolarization of the resting membrane potential, increase excitability and altered Kir current density in NAc-MSN (3). Recently, it was reported that the sub-threshold voltage-dependent conductances are critical to both the membrane excitability state and synaptic integration via regulation of both resting membrane potential (RMP) and the action potential (AP) we first investigate the AP shape. We found that VPA-prenatal exposure decreases both AP timing and AHP and increases AP amplitude in VPA-exposed rats, that could be causes of sub-threshold voltage-dependent conductances. Moreover VPA affected excitatory neurotransmission suggesting a post-synaptic changes in glutamatergic receptors. Finally we evaluated the possible age-dependent effect of VPA-prenatal exposure and surprisingly we found that in adulthood there was normalization of neuronal excitability in line with a repolarization of RMP and increase of AP threshold. Moreover VPA induced an opposite effect on synaptic neurotransmission in adult VPA-exposed rats. Our results suggest that VPA induces a time dependent changes in NAc-MSN. Our present study demonstrate that VPA-prenatal exposure induce a neurodevelopmental perturbations in corticolimbic structures involved in social and reward processes suggesting a new link between NAc-MSN dysfunction and autistic core features. Furthermore our study identify NAc-MSN like a new therapeutic target in ASD treatment.

- **References**

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