eNeuro

Research Article: New Research | Disorders of the Nervous System

Maternal exposure to the cannabinoid agonist WIN 55,12,2 during lactation induces lasting behavioral and synaptic alterations in the rat adult offspring of both sexes

https://doi.org/10.1523/ENEURO.0144-20.2020

Cite as: eNeuro 2020; 10.1523/ENEURO.0144-20.2020

Received: 10 April 2020
Revised: 9 July 2020
Accepted: 27 July 2020

Manuscript Title Page

1. Manuscript Title (50 word maximum)

Maternal exposure to the cannabinoid agonist WIN 55,12,2 during lactation induces lasting behavioral and synaptic alterations in the rat adult offspring of both sexes

2. Abbreviated Title (50 character maximum)

Lasting effects of cannabinoid during lactation

3. List all Author Names and Affiliations in order as they would appear in the published article

Andrew F Scheyer[1,2,3], Milene Borsoi[1,2,3], Anne-Laure Pelissier-Alicot[1,2,3,4,5], Olivier JJ Manzoni[1,2,3]

[1] INMED, INSERM U1249, Marseille, France.
[2] Aix-Marseille University, France.
[3] Cannalab Cannabinoids Neuroscience Research International Associated Laboratory. INSERM-Aix-Marseille University/Indiana University.
[4] APHM, CHU Conception, Service de Psychiatrie, 13005 Marseille, France.
[5] APHM, CHU Timone Adultes, Service de Médecine Légale, 13005 Marseille, France.

4. Author Contributions:

A.S., M.B., A.L.P and O.J.M. designed research; A.S. and M.B. performed research; A.S. and M.B. analyzed data; A.S. and O.J.M wrote the paper.

5. Correspondence should be addressed to (include email address)
Olivier Manzoni, PhD.
INMED, INSERM U1249
Parc Scientifique de Luminy
BP13 – 13273 Marseille cedex 09, France.
Email : olivier.manzoni@inserm.fr

6. Number of Figures 5

7. Number of Tables 8

8. Number of Multimedia 0

9. Number of words for Abstract 148

10. Number of words for Significance Statement 98

11. Number of words for Introduction 273

12. Number of words for Discussion 823

13. Acknowledgements

The authors are grateful to the Chavis-Manzoni team members for helpful discussions.

14. Conflict of Interest

A. Authors report no conflict of interest

15. Funding sources

This work was supported by the Institut National de la Santé et de la Recherche Médicale (INSERM); the INSERM-NIH exchange program (to A.F.S.); Fondation pour la Recherche Médicale (Equipe FRM 2015 to O.M.) and the NIH (R01DA043982 to O.M.).

Maternal exposure to the cannabinoid agonist WIN 55,12,2 during lactation induces lasting behavioral and synaptic alterations in the rat adult offspring of both sexes

Abstract

Consumption of cannabis during pregnancy and the lactation period is a rising public health concern (Scheyer et al. 2019b). Exposure to synthetic or plant-derived cannabinoids via lactation disrupts the development of GABAergic neurons in the prefrontal cortex and alters early-life behaviors (Scheyer et al. 2020b). Recently, additional data revealed that ∆9-tetrahydrocannabinol (THC) perinatal exposure via lactation causes lasting behavioral and neuronal consequences (Scheyer et al. 2020a).

Here, the long-term effects in adult offspring of maternal exposure to the synthetic cannabinoid agonist WIN 55,12,2 (WIN) are reported. The data demonstrate that rats exposed during lactation to WIN display social and motivational deficits at adulthood. These behavioral changes were paralleled by a specific loss of endocannabinoid-mediated long-term depression in the prefrontal cortex and nucleus accumbens, while other forms of synaptic plasticity remained intact. Thus, similarly to THC, perinatal WIN exposure via lactation induces behavioral and synaptic abnormalities lasting into adulthood.

Significance Statement

Consumption of cannabis during pregnancy and the lactation period is a rising public health concern. Exposure to synthetic or plant-derived cannabinoids via lactation disrupts perinatal programming in the prefrontal cortex and early-life behaviors. Here, we explored the long-term effects of maternal exposure to the synthetic cannabinoid agonist WIN 55,12,2 in the adult offspring. The results indicate that rats exposed during lactation to WIN display social and motivational deficits at adulthood. These behavioral changes were paralleled by a specific loss of endocannabinoid-mediated long-term depression in the prefrontal cortex and nucleus accumbens, while other forms of synaptic plasticity remained intact.

Introduction

Cannabis consumption by pregnant women is progressively increasing (Scheyer et al. 2019b; Hurd et al. 2019). The principle psychoactive component of cannabis, ∆9-tetrahydrocannabinol (THC), in addition to other cannabinoids, is actively transferred to the developing infant via breastfeeding (Scheyer et al. 2019a; Hurd et al. 2019). During the perinatal period (i.e. during prenatal and early postnatal development), the developing brain is acutely sensitive to exogenous cannabinoids (Scheyer et al. 2019a). Exposure to cannabinoids via lactation alters the developmental trajectory of the prefrontal cortex (PFC), which has been identified as a cortical hub essential to planning, cognitive flexibility and emotional behaviors (Goldman-Rakic 1991) and a common target in various endocannabinoid-related synaptopathies (Araque et al. 2017). Thus, exposure of lactating females to either THC, or a synthetic agonist of CB1R, altered the maturational trajectory of GABAergic transmission and led to behavioral abnormalities in early life (Scheyer et al. 2020b). Disruptions to GABAergic development are known to occur following adolescent exposure to THC, as well (Renard et al. 2017). Furthermore, perinatal THC exposure via lactation elicits lasting, deleterious impacts on social behavior and synaptic plasticity in the PFC of the adult offspring (Scheyer et al. 2020a).

Here, we investigated the effects in both sexes of adult offspring of maternal exposure to the synthetic cannabinoid agonist, WIN 55,12,2 (WIN). These data demonstrate that rats exposed during lactation to WIN display social and motivational deficits at adulthood. These behavioral changes were paralleled by a specific loss of endocannabinoid-mediated long-term depression in the PFC and nucleus accumbens, while other forms of synaptic plasticity remained intact. Thus, perinatal WIN exposure via lactation induces behavior and synaptic abnormalities lasting into adulthood.

Materials and Methods

Animals

Animals were treated in compliance with the European Communities Council Directive (86/609/EEC) and the United States NIH Guide for the Care and Use of Laboratory Animals. All animal procedures were performed in accordance with the [Author University] animal care committee's regulations. All rats were group-housed with 12h light/dark cycles with ad libitum access to food and water (Zeitgeber Time ZT0=7a.m). All behavioral, biochemical and synaptic plasticity experiments were performed on male and female RjHan:wi-Wistar rats (>P90) from pregnant females obtained from Janvier Labs. Pregnant dams arrived at E15 and remained undisturbed until delivery. Newborn litters found before 05:00p.m. were considered to be born that day (P0). Dams were injected daily subcutaneously (s.c.) from P01-10 with WIN (0.5 mg/kg/day), dissolved in 10% polyethylene glycol/10% Tween/80% saline and injected subcutaneously (Borsoi et al. 2019). Control dams (Sham) received vehicle.

Behavioral procedures

Open field: Observations were conducted after rats were adapted to the room laboratory conditions for at least 1 h prior to testing. Tests were conducted in a 45 × 45 cm transparent Plexiglass arena. All behavioral procedures were performed between 10:00 am and 3:00 pm. A video tracking system (Ethovision XT, Noldus Information Technology) recorded the total distance traveled and time spent in the central zone (21 × 21 cm) of the apparatus (Borsoi et al. 2019).

Social interaction: The apparatus consisted of a transparent acrylic chamber (120 x 80 cm) divided into three equal compartments (40 cm each) partially separated by white walls. The central compartment was empty and lateral compartments had an empty wire cage (20 cm diameter) were an object or a new rat (social stimulus) were placed during the test. WIN or sham-exposed rats were individually habituated to the test cage containing the two empty wire cages for 5 min immediately prior to testing. The first trial (social approach, 5 min duration) consisted of giving the tested rat the option to socialize with either a novel object or a new, naïve, age- and sex-mate conspecific rat that were placed into the wire cages positioned on the arena's opposite sides. Thirty minutes later, the tested rat returned to the apparatus for the second trial (social memory, 5min duration) wherein the two compartments held either the now-familiar rat from the first testing phase or a second, previously unknown, naïve, age- and sex-mate conspecific. Only rats with no compartment preference during the habituation phase were used. Time spent in each compartment and time spent exploring wire cages during the social approach and social memory phases were scored. Social Preference Ratio was calculated as time spent exploring either the wire cage containing the object, or the new rat divided by total time exploring both wire cages. Likewise, Social Memory Ratio was calculated as time spent exploring either the wire cage containing the rat used in the first trial or the new rat divided by total time exploring both wire cages. Recognition index higher than 0.5 indicates preferable object recognition memory.

Anhedonia: We performed sucrose consumption tests (Monleon et al. 1995; Bessa et al. 2009). Rats were exposed for 24 h to a bottle containing a sucrose solution (5% in tap water, Sigma), placed in the wire-top cage cover adjacent to standard tap water, followed by 12 h of water deprivation and a 20 minute exposure to two identical bottles (one filled with 5% sucrose solution and the other with water). Bottles were placed at opposite ends of the cage and counterbalanced across groups to avoid side bias. Sucrose preference was calculated as the ratio of the volume of sucrose versus volume or consumed during the 20-minute test. All animals were habituated to the testing room 24 h prior to initiating the sucrose preference test.

Slice preparation

Adult male and female rats were anesthetized with isoflurane and sacrificed (Bara et al. 2018; Borsoi et al. 2019). The brain was sliced (300 µm) in the coronal plane with a vibratome (Integraslice, Campden Instruments) 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 medial prefrontal cortex (PFC) or the nucleus accumbens (NAc) were stored for 1 hr 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 NaH2PO_4, and were equilibrated with 95% O2/5% CO_2 and then at room temperature until the time of recording. During the recording, slices were placed in the recording chamber and superfused at 2 ml/min with low Ca^{2+} or normal Ca^{2+} ACSF (PFC and NAc respectively). All experiments were done at 32°C (PFC) or 25°C (NAc). The superfusion medium contained picrotoxin (100 mM) to block gamma-aminobutyric acid types A (GABA-A) receptors. All drugs were added at the final concentration to the superfusion medium.

Electrophysiology

Whole cell patch-clamp of visualized layer five pyramidal medial PFC or medium spiny neurons and field potential recordings were made in coronal slices using standard procedures (Bara et al. 2018; Borsoi et al. 2019). Neurons were visualized using an upright microscope with infrared illumination. The intracellular solution was based on K+ gluconate (in mM: 145 K^+ gluconate, 3 NaCl, 1 $MgCl_2$, 1 EGTA, 0.3 $CaCl_2$, 2 Na^{2+} ATP, and 0.3 Na^+ GTP, 0.2 cAMP, buffered with 10 HEPES). The pH was adjusted to 7.2 and osmolarity to 290–300 mOsm. Electrode resistance was 4–6 MOhms. Recordings were performed with an Axopatch-200B amplifier. Data were low pass filtered at 2kHz, digitized (10 kHz, DigiData 1440A, Axon Instrument), collected using Clampex 10.2 and analyzed using Clampfit 10.2 (all from Molecular Device, Sunnyvale, USA).

A -2 mV hyperpolarizing pulse was applied before each evoked EPSC in order to evaluate the access resistance and those experiments in which this parameter changed >25% were rejected. Access resistance compensation was not used, and acceptable access resistance was <30 MOhms. The potential reference of the amplifier was adjusted to zero prior to breaking into the cell. Cells were held at -75mV.

Current-voltage (I-V) curves were made by a series of hyperpolarizing to depolarizing current steps immediately after breaking into the cell. Membrane resistance was estimated from the I–V curve around resting membrane potential (Martin et al. 2015). Field potential recordings were made in coronal slices containing the PFC or the NAc (Kasanetz et al. 2013). During the recording, slices were placed in the recording

chamber and superfused at 2 ml/min with low Ca^{2+} ACSF. All experiments were done at 32°C. The superfusion medium contained picrotoxin (100 mM) to block GABA Type A (GABA-A) receptors. All drugs were added at the final concentration to the superfusion medium. The glutamatergic nature of the field EPSP (fEPSP) was systematically confirmed at the end of the experiments using the ionotropic glutamate receptor antagonist CNQX (20 mM), which specifically blocked the synaptic component without altering the non-synaptic.

Both fEPSP area and amplitude were analyzed. Stimulation was performed with a glass electrode filled with ACSF and the stimulus intensity was adjusted ~60% of maximal intensity after performing an input–output curve (baseline EPSC amplitudes ranged between 50 and 150 pA). Stimulation frequency was set at 0.1 Hz.

Data acquisition and analysis

The magnitude of plasticity was calculated at 0-10min and 30–40 min after induction (for TBS-LTP and eCB-LTD) or drug application (mGlu2/3-LTD) as percentage of baseline responses. Statistical analysis of data was performed with Prism (GraphPad Software) using tests indicated in the main text after outlier subtraction (Grubb's test, alpha level 0.05). All values are given as mean ±SEM, and statistical significance was set at $p<0.05$.

Results

In rodent models, exposure to cannabinoids (both synthetic and plant-derived) during gestation or early development induces an array of deleterious consequences on behavior manifesting both at early life and adulthood (Scheyer et al. 2019a; Hurd et al. 2019).

Perinatal exposure via lactation to either the plant-derived phytocannabinoid ∆9-tetrahydrocannabinol (THC), or the synthetic cannabinoid, WIN 55,212-2 (WIN), induces a significant delay in the trajectory of GABAergic development in the PFC of developing offspring, an effect which is accompanied by substantial behavioral alterations (Scheyer et al. 2020b). Further, the progeny of dams similarly exposed via lactation to THC during the first 10 days of postnatal life exhibit lasting deficits in synaptic plasticity in the PFC as well as augmented social behavior at adulthood (Scheyer et al. 2020a).

Here, we used this same protocol of perinatal cannabinoid exposure in order to determine if synaptic and behavioral consequences are similarly produced following maternal administration of WIN. Thus, lactating dams were treated with a low dose of WIN (0.5 mg/kg, s.c.) or its vehicle (herein referred to as Sham) from postnatal day 1 to 10 (PND 1-10). Experiments were then conducted in the male and female offspring at adulthood (>PND90).

All treatment effects (e.g. Sham vs WIN) were found to be consistent across sexes. Thus, for figures and statistical analyses, data for male and female rats within treatment condition were combined. However, differences between the sexes within treatment conditions were noted in some measures. Details of within-treatment sex differences can be found in tables 1-8.

Perinatal exposure to a synthetic cannabimimetic alters social behavior and memory at adulthood.

In order to determine if the behavioral repertoire of WIN-exposed animals is altered at adulthood, we performed several behavioral analyses in both male and female rats. Because perinatally THC-exposed animals exhibited augmented social behavior at adulthood, we initiated a social approach and memory assay (Figure 1a-d; Table 1).

First, during the social approach portion of the assay, WIN-exposed animals exhibited significantly heightened preference for a novel rat over a novel object, as compared to Sham rats (Figure 1a). Both Sham- and WIN-exposed rats exhibited a significant preference for the social stimulus as compared to the object (Figure 1b). However, the magnitude of difference between time spent exploring the novel rat versus the novel object was significantly heightened in the adult offspring of WIN-treated dams. During the subsequent memory test, both Sham- and WIN-exposed rats exhibited a similar social preference for a novel rat over the familiar rat from the social approach assay (Figure 1c-d).

Further, naturalistic behavior was observed prior to the social approach/memory testing by observing animals in the open field assay. No significant differences were noted in the time spent in the center of the arena, distance covered during the trial, or the exploratory behavior (# of rearing events) during the open field test (Figure 1e-g; Table 2).

Perinatal exposure to WIN alters prefontal synaptic plasticity at adulthood.

Perinatal THC exposure alters several forms of synaptic plasticity in the PFC at adulthood (Scheyer et al. 2020a). Thus, we elected to examine three forms of PFC plasticity in order to determine if similar alterations followed perinatal WIN exposure. First, we used a 10-minute, 10Hz stimulation of superficial layers of the PFC in order to elicit an endocannabinoid-dependent long-term depression (eCB-LTD) at deep layer synapses (Figure 2a-b; Table 3).

Here, we found that while Sham-exposed rats exhibited robust, lasting depression 30-40-minutes following the 10-minute protocol, no such LTD was observed in the PFC of WIN-exposed rats. This finding is in line with our previous data showing an ablation of eCB-LTD in the PFC of THC-exposed rats.

Next, we examined a distinct form of LTD in the PFC mediated by mGlu2/3 receptors (Bara et al. 2018) which has previously been shown to be disrupted by chronic exposure to drugs (Hoffman et al. 2003; Huang et al. 2007; Kasanetz et al. 2013) and augmented at adulthood following perinatal THC exposure (Scheyer et al. 2020a). Thus, we exposed acute PFC slices to the mGlu2/3 agonist LY379268 (300nM) in order to elicit an mGlu2/3-dependent LTD (Figure 2c-d; Table 3). Here, we found that PFC synapses in slices obtained from the offspring of both Sham- and WIN-treated dams exhibited a similar magnitude of mGlu2/3-dependent LTD at 30-40 minutes following drug application.

Finally, we used a theta-burst stimulation protocol at superficial layers of the PFC in order to induce a lasting synaptic potentiation (TBS-LTP) at deep layer synapses. Here, we found no alterations to the time-course or magnitude of plasticity between slices obtained from the adult offspring of Sham-, as compared to WIN-treated dams (Figure 2e-f; Table 4). Of note, these results stand in contrast to those from THC-exposed rats, wherein TBS-LTP is impaired at adulthood (Scheyer et al. 2020a).

Because perinatal THC exposure altered parameters of cell excitability in the PFC at adulthood, we next sought to determine if WIN exposure elicited similar augmentations in excitability. Interestingly, pyramidal neurons in PFC slices obtained from the adult offspring of Sham- and WIN-treated dams did not differ with regards to input-output excitability, spikes elicited by progressive current injections, nor in the rheobase or resting membrane potential (Figure 3a-d; Table 5).

Perinatal exposure to WIN alters synaptic plasticity and cellular properties in the accumbens at adulthood.

Recent data have demonstrated that cannabinoids, experimenter- or self-administered, abolish LTD in the NAc (Mato et al. 2004, 2005; Neuhofer and Kalivas 2018; Spencer et al. 2018). Thus, we sought to determine if perinatal WIN exposure elicited similar deficits in LTD in the NAc at adulthood. We found that while the adult offspring of Sham-treated dams exhibited robust LTD 30-40 minutes after a 10-minute, 10Hz stimulation, no such effect was found in the NAc of WIN-exposed rats (Figure 4a-b; Table 6). Interestingly, unlike in the PFC, these alterations were accompanied by a significant reduction in the resting membrane potential of the principal neurons of the NAc, medium spiny neurons (MSN; Figure 4f). No other parameters of cell excitability were found modified comparing MSNs in slices obtained from Sham-, as compared to WIN-exposed rats (Figure 4c-e; Table 7).

Perinatal exposure to WIN enhances sucrose consumption at adulthood.

The NAc plays an important role in reward-associated behavior, and recent data indicate a relationship between LTD in the NAc and reward-seeking behavior including sucrose consumption (Bobadilla et al. 2017; Bilbao et al. 2020). Thus, we examined the magnitude of sucrose preference in a two-bottle choice paradigm in the adult offspring of Sham- and WIN-treated dams. Here, we found that while both groups exhibited a preference for a 5% sucrose solution (as compared to plain water) and consumed similar total quantities of liquid during the test (Figure 5a; Table 8), the ratio of sucrose/water consumption was significantly higher in WIN- as compared to Sham-treated adult offspring (Figure 5b; Table 8). Thus, in addition to alterations to synaptic plasticity and intrinsic excitability of MSNs in the NAc, perinatal WIN exposure enhances reward-seeking behavior at adulthood.

Discussion

Here, using the synthetic cannabinoid WIN, we found that exposure to plant-derived phytocannabinoid THC via lactation induces behavioral and electrophysiological alterations lasting into adulthood. Specifically, we found altered social behavior, memory, and eCB-mediated synaptic plasticity in the PFC of adult offspring of dams administered WIN during the first 10 days of postnatal life. We also showing synaptic deficits and cellular alterations in the NAc along with enhanced sucrose preference, indicative of heightened reward seeking in WIN-exposed adults.

First, our behavioral analyses revealed that perinatal WIN exposure augments social preference in the adult offspring of WIN-treated dams. This result confirms the social augmentation seen following perinatal THC exposure (Scheyer et al. 2020a) but diverge from the effects of in utero THC exposure (i.e. social exploration was reduced). Such discrepancies point to potential differences in the sensitivity of developmental windows through the prenatal and early postnatal periods.

We also report that WIN exposure does not affect social memory, WIN abolishes novel object recognition at adulthood. Interestingly, social approach and memory is a complex behavior collating activity from diverse brain regions governing motivation and reward such as the amygdala (Adolphs 2001) and nucleus accumbens (Dölen et al. 2013). Indeed, augmentations in social approach behavior are often associated with decreased amygdalar function and signaling in the nucleus accumbens, where oxytocin-mediated transmission is a key regulator of social approach and reward (Dölen et al. 2013), and is itself governed by the ECS (Wei et al. 2015). Previous data have highlighted the role of CCK interneuron dysfunction in WIN-mediated disruptions to social interaction (Vargish et al. 2017), a possible contributor to aberrant social behavior seen here that requires further investigation. Thus, variable impacts on memory and exploration behavior are likely attributable to underlying differences in the driving circuitry.

Results from the current study examining the long-term consequences of perinatal WIN exposure adds to a preliminary report of dysfunctional eCB-LTD in the PFC of perinatally THC exposed offspring (Scheyer et al. 2020a). In contrast with THC treatment however, perinatal WIN did not lead to an enhanced magnitude of mGlu2/3-LTD nor a loss of TBS-LTP in the WIN-exposed progeny at adulthood. Differences in the pharmacokinetics, bioavailability and pharmacological profiles of WIN and THC may explain these differences (Pertwee 2005). Indeed, while WIN is a highly selective agonist of CB1, THC exhibits a diverse range of activity from partial agonist targeting of CB1 and CB2 to activation of several transient receptor potential channels, orphan receptors, and the nuclear PPARγ. Despite these subtle differences, these data and those from previous studies suggest that alteration of PFC synaptic plasticity and social behavior at adulthood are common endophenotypes of perinatal cannabinoid exposure (Hoffman et al. 2003; Vargish et al. 2017; Bara et al. 2018; Scheyer et al. 2020b, a).

Perinatal THC exposure decreases excitability of principle neurons of the PFC (Scheyer et al. 2020a) in a fashion similar to chronic adolescent THC exposure in mice (Pickel et al. 2019). Here, we found that no such differences followed perinatal WIN exposure. These data point to a dissociation between measures of intrinsic excitability and synaptic plasticity within the PFC, as changes in these domains appear independent. Thus, alternative explanations for the loss of eCB-LTD must be considered

in light of a lack of changes to cell-excitability, including alterations to receptor function or other changes to the ECS such as alterations in eCB tone.

The NAc is essential to reward-associated behavior and eCB-mediated LTD in the NAc core controls reward-seeking behavior (Bilbao et al. 2020). Here, we found that this eCB-LTD is ablated in the NAc of the adult offspring of WIN-treated dams. This finding is in line with multiple reports of altered LTD in the NAc of cannabinoid-exposed animals (Mato et al. 2004, 2005; Neuhofer and Kalivas 2018; Spencer et al. 2018). In contrast with our recordings in PFC principal neurons, we observed a significant reduction in the resting membrane potential of NAc MSNs. Further, in examining the reward-seeking behavior of these WIN-exposed offspring, we also found that the ratio of sucrose/water consumption in the two-bottle choice task was significantly higher in WIN- as compared to Sham-treated adult offspring. Thus, in the NAc of WIN-exposed progeny, the loss of eCB-LTD and associated cell-excitability modifications were paralleled by modifications of reward-seeking behavior at adulthood.

In conclusion, these results indicate that perinatal exposure via lactation to a synthetic cannabinoid reproduces some of the long-lasting deficits induced at multiple scales by THC. Augmented social behavior and a loss of eCB-LTD in the PFC are therefore similar consequences of perinatal exposure to both naturally occurring phytocannabinoids and synthetic cannabimimetics. Additionally, we found that WIN exposure ablates eCB-LTD in the NAc, where the resting membrane potential of MSNs was found to be significantly decreased. These findings may indeed correlate with an enhanced sucrose-preference amongst WIN-exposed offspring. Together, these findings further illustrate the vulnerability of the developing brain and, consequently, behavior, to early-life insults to the endocannabinoid system via exposure to cannabinoid agonists.

Author contributions: A.S., M.B., A.L.P and O.J.M. designed research; A.S. and M.B. performed research; A.S. and M.B. analyzed data; A.S. and O.J.M wrote the paper. The authors declare no conflict of interest.

Declarations of interest: The authors declare no competing interests.

References

Adolphs R (2001) The neurobiology of social cognition. Curr. Opin. Neurobiol. 11:231–239

Araque A, Castillo PE, Manzoni OJ, Tonini R (2017) Synaptic functions of endocannabinoid signaling in health and disease. Neuropharmacology 124:13–24

Bara A, Manduca A, Bernabeu A, et al (2018) Sex-dependent effects of in utero cannabinoid exposure on cortical function. Elife 7:. https://doi.org/10.7554/eLife.36234

Bessa JM, Mesquita AR, Oliveira M, et al (2009) A trans-dimensional approach to the behavioral aspects of depression. Front Behav Neurosci 3:. https://doi.org/10.3389/neuro.08.001.2009

Bilbao A, Neuhofer D, Sepers M, et al (2020) Endocannabinoid LTD in Accumbal D1 Neurons Mediates Reward-Seeking Behavior. iScience 23:. https://doi.org/10.1016/j.isci.2020.100951

Bobadilla AC, Garcia-Keller C, Heinsbroek JA, et al (2017) Accumbens mechanisms for cued sucrose seeking. Neuropsychopharmacology 42:2377–2386. https://doi.org/10.1038/npp.2017.153

Borsoi M, Manduca A, Bara A, et al (2019) Sex differences in the behavioral and synaptic consequences of a single In vivo exposure to the synthetic cannabimimetic win55,212-2 at puberty and adulthood. Front Behav Neurosci 13:. https://doi.org/10.3389/fnbeh.2019.00023

Dölen G, Darvishzadeh A, Huang KW, Malenka RC (2013) Social reward requires coordinated activity of nucleus accumbens oxytocin and serotonin. Nature 501:179–184. https://doi.org/10.1038/nature12518

Goldman-Rakic PS (1991) Chapter 16 Cellular and circuit basis of working memory in prefrontal cortex of nonhuman primates. Prog Brain Res 85:325–336. https://doi.org/10.1016/S0079-6123(08)62688-6

Hoffman AF, Oz M, Caulder T, Lupica CR (2003) Functional tolerance and blockade of long-term depression at synapses in the nucleus accumbens after chronic cannabinoid exposure. J Neurosci 23:4815–4820. https://doi.org/10.1523/jneurosci.23-12-04815.2003

Huang CC, Yang PC, Lin HJ, Hsu K Sen (2007) Repeated cocaine administration impairs group II metabotropic glutamate receptor-mediated long-term depression in rat medial prefrontal cortex. J Neurosci 27:2958–2968. https://doi.org/10.1523/JNEUROSCI.4247-06.2007

Hurd YL, Manzoni OJ, Pletnikov M V., et al (2019) Cannabis and the Developing Brain: Insights into Its Long-Lasting Effects. J Neurosci 39:8250–8258. https://doi.org/10.1523/JNEUROSCI.1165-19.2019

Kasanetz F, Lafourcade M, Deroche-Gamonet V, et al (2013) Prefrontal synaptic markers of cocaine addiction-like behavior in rats. Mol Psychiatry 18:729–737. https://doi.org/10.1038/mp.2012.59

Martin HGS, Bernabeu A, Lassalle O, et al (2015) Endocannabinoids mediate muscarinic acetylcholine receptor-dependent long-term depression in the adult medial prefrontal cortex. Front Cell Neurosci 9:1–11. https://doi.org/10.3389/fncel.2015.00457

Mato S, Chevaleyre V, Robbe D, et al (2004) A single in-vivo exposure to Δ9THC blocks endocannabinoid-mediated synaptic plasticity. Nat Neurosci 7:585–586. https://doi.org/10.1038/nn1251

Mato S, Robbe D, Puente N, et al (2005) Presynaptic homeostatic plasticity rescues long-term depression after chronic Δ9-tetrahydrocannabinol exposure. J Neurosci 25:11619–11627. https://doi.org/10.1523/JNEUROSCI.2294-05.2005

Monleon S, Parra A, Simon VM, et al (1995) Attenuation of sucrose consumption in mice by chronic mild stress and its restoration by imipramine. Psychopharmacology (Berl) 117:453–457. https://doi.org/10.1007/BF02246218

Neuhofer D, Kalivas P (2018) Metaplasticity at the addicted tetrapartite synapse: A common denominator of drug induced adaptations and potential treatment target for addiction. Neurobiol Learn Mem 154:97–111. https://doi.org/10.1016/j.nlm.2018.02.007

Pertwee RG (2005) Pharmacological actions of cannabinoids. Handb Exp Pharmacol 168:1–51. https://doi.org/10.1007/3-540-26573-2-1

Pickel VM, Bourie F, Chan J, et al (2019) Chronic adolescent exposure to ∆9-tetrahydrocannabinol decreases NMDA current and extrasynaptic plasmalemmal density of NMDA GluN1 subunits in the prelimbic cortex of adult male mice. Neuropsychopharmacology. https://doi.org/10.1038/s41386-019-0466-9

Renard J, Szkudlarek HJ, Kramar CP, et al (2017) Adolescent THC Exposure Causes Enduring Prefrontal Cortical Disruption of GABAergic Inhibition and Dysregulation of Sub-Cortical Dopamine Function. Sci Rep 7:11420. https://doi.org/10.1038/s41598-017-11645-8

Scheyer AF, Borsoi M, Pelissier- Alicot A-L, Manzoni OJJ (2020a) Perinatal THC exposure via lactation induces lasting alterations to social behavior and prefrontal cortex function in rats at adulthood. Neuropsychopharmacology. https://doi.org/10.1038/s41386-020-0716-x

Scheyer AF, Borsoi M, Wager-Miller J, et al (2020b) Cannabinoid Exposure via Lactation in Rats Disrupts Perinatal Programming of the Gamma-Aminobutyric Acid Trajectory and Select Early-Life Behaviors. Biol Psychiatry 87:666–677. https://doi.org/10.1016/j.biopsych.2019.08.023

Scheyer AF, Melis M, Trezza V, Manzoni OJJ (2019a) Consequences of Perinatal Cannabis Exposure. Trends Neurosci. https://doi.org/10.1016/j.tins.2019.08.010

Scheyer AF, Melis M, Trezza V, Manzoni OJJ (2019b) Consequences of Perinatal Cannabis Exposure. Trends Neurosci 42:871–884. https://doi.org/10.1016/j.tins.2019.08.010

Spencer S, Neuhofer D, Chioma VC, et al (2018) A Model of Δ9-Tetrahydrocannabinol Self-administration and Reinstatement That Alters Synaptic Plasticity in Nucleus Accumbens. Biol Psychiatry 84:601–610. https://doi.org/10.1016/j.biopsych.2018.04.016

Vargish GA, Pelkey KA, Yuan X, et al (2017) Persistent inhibitory circuit defects and disrupted social behaviour following in utero exogenous cannabinoid exposure. 22:56–67. https://doi.org/10.1038/mp.2016.17

Wei D, Lee DY, Cox CD, et al (2015) Endocannabinoid signaling mediates oxytocin-driven social reward. Proc Natl Acad Sci U S A 112:14084–14089. https://doi.org/10.1073/pnas.1509795112

Figure Legends

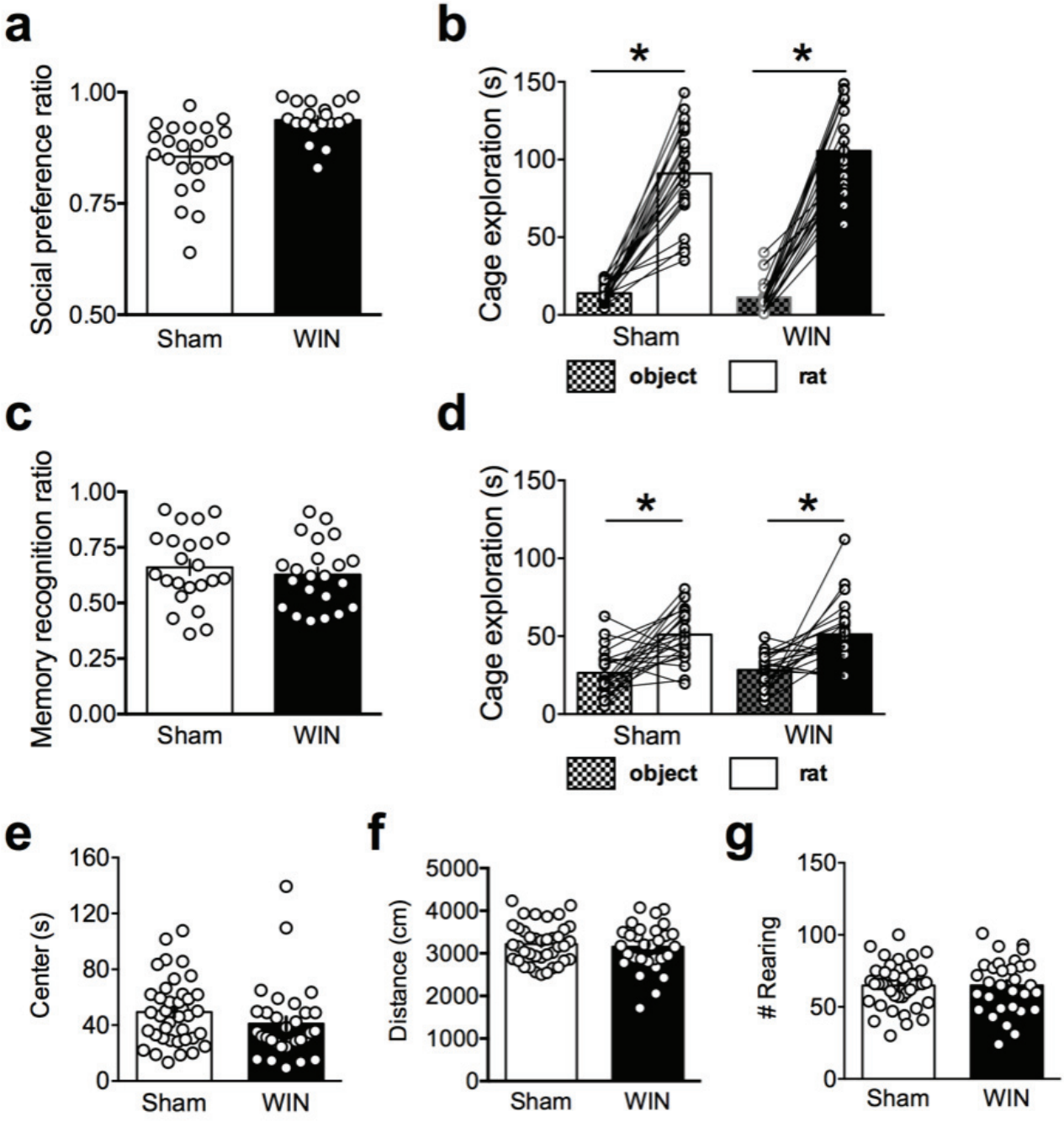
Figure 1. Perinatal WIN alters social approach, but not social memory behavior, nor behavior in the open field environment.
a: Adult offspring of WIN-treated dams exhibit significantly higher social preference than those of Sham-treated dams (Two-tailed T-test, P = 0.0001; N = 23, 19 respectively). **b**: Time spent exploring a novel rat is significantly higher than time spent exploring a novel object for both Sham- and WIN-exposed rats (One-way ANOVA, $F_{3,86}$ = 14.49; Tukey's post-hoc analysis, P<0.0001 for both groups; N = 23, 19 respectively). **c,d**: In the subsequent social memory test, the adult offspring of both Sham- and WIN-treated dams exhibited significantly higher preference for a novel, as compared to familiar, rats. **c**: The social memory index does not differ between the two groups (Two-tailed T-test, P = 0.557). **d**: Time spent exploring a novel rat is significantly higher than time spent exploring a familiar rat for both Sham- and WIN-exposed rats (One-way ANOVA, $F_{3,86}$ = 2.137; Tukey's post-hoc analysis, P<0.0001 for both groups). **e-g**: Behavior in the open field environment does not differ between the offspring of Sham- and WIN-treated dams (N = 39, 31 respectively). Time spent in the center of the arena, total distance covered, and the number of rearing events is not significantly different between groups (Two-tailed T-tests, P = 0.1817, 0.5991 and 0.9783, respectively). *P<0.05

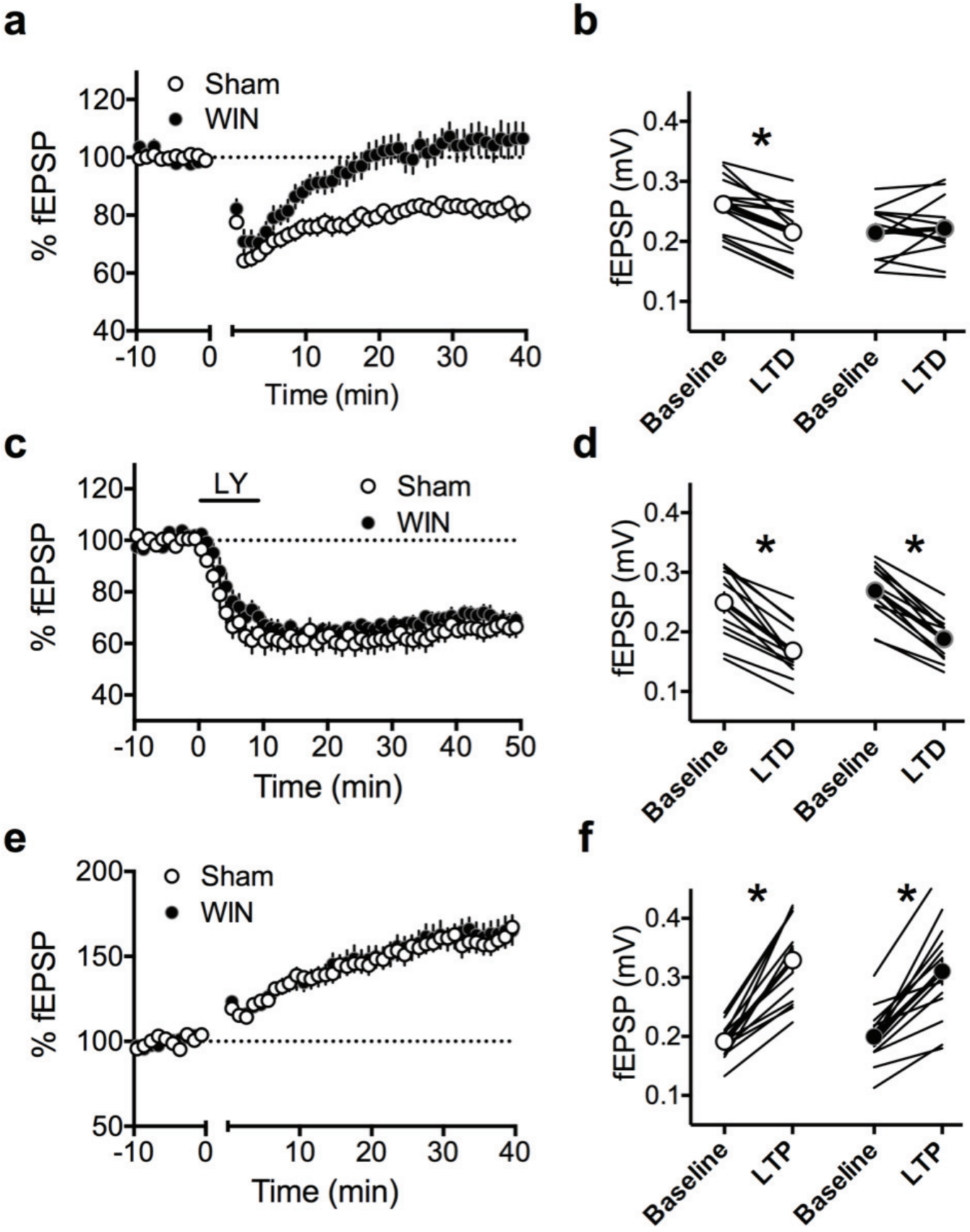
Figure 2. Perinatal WIN exposure induces a selective deficit in LTD in the PFC of adult offspring.
a: A 10-minute, 10Hz field stimulation of layer 2/3 cells in the PFC of the adult offspring of Sham-treated dams (N = 14) elicited a robust eCB-LTD at deep layer synapses. However, this same protocol failed to induce eCB-LTD in the adult offspring of dams treat with WIN (N = 14). **b**: fEPSP magnitude at baseline (-10 to 0 minutes) and LTD (35-40 minutes post-tetanus) values corresponding to the normalized values in **a** (Two-way RM ANOVA, $F_{1,24}$ = 16.58, P = 0.0004. Sidak's multiple comparisons test, P=0.0332 and 0.9412, respectively). **c**: LTD mediated by $mGlu_{2/3}$ receptors (mGluR-LTD) is not altered in WIN-exposed offspring. mGluR-LTD, induced via a 10-minute application of LY379268 (LY; 30nM), produced a significant depression at deep layer synapses of the PFC in the adult offspring of both sham- and WIN-treated dams (N = 12 and 12, respectively). **d**: fEPSP magnitude at baseline (-10 to 0 minutes) and LTD (30-40 minutes post-drug) values corresponding to the normalized values in **c.** No differences were found between groups comparing the ten-minute baseline period and the last ten minutes of recording, however both groups exhibited a significant difference of fEPSP magnitude at baseline (i.e. -10 to 0 minutes) as compared to 30-40 minutes post-drug (Two-Way RM ANOVA, F1,11 = 96.69, P<0.0001. Sidak's multiple comparisons test, P<0.0001 for both groups). **e**: A TBS protocol (5 pulses at 100hz, repeated 4 times) at layer 2/3 cells in the PFC of the adult offspring of both Sham- and WIN-treated dams elicited a robust LTP at deep layer synapses (N = 13, 15 respectively). **f**: fEPSP magnitude at baseline (-10 to 0 minutes) and LTP (30-40 minutes post-TBS) values corresponding to the normalized values in **e.** Both groups exhibited significant differences between the fEPSP magnitude at 30-40 minutes as compared to -10 to 0 minutes (Two-way RM ANOVA, F1,25 = 1.737. Sidak's multiple comparisons test, P<0.0001 for both groups). *P<0.05

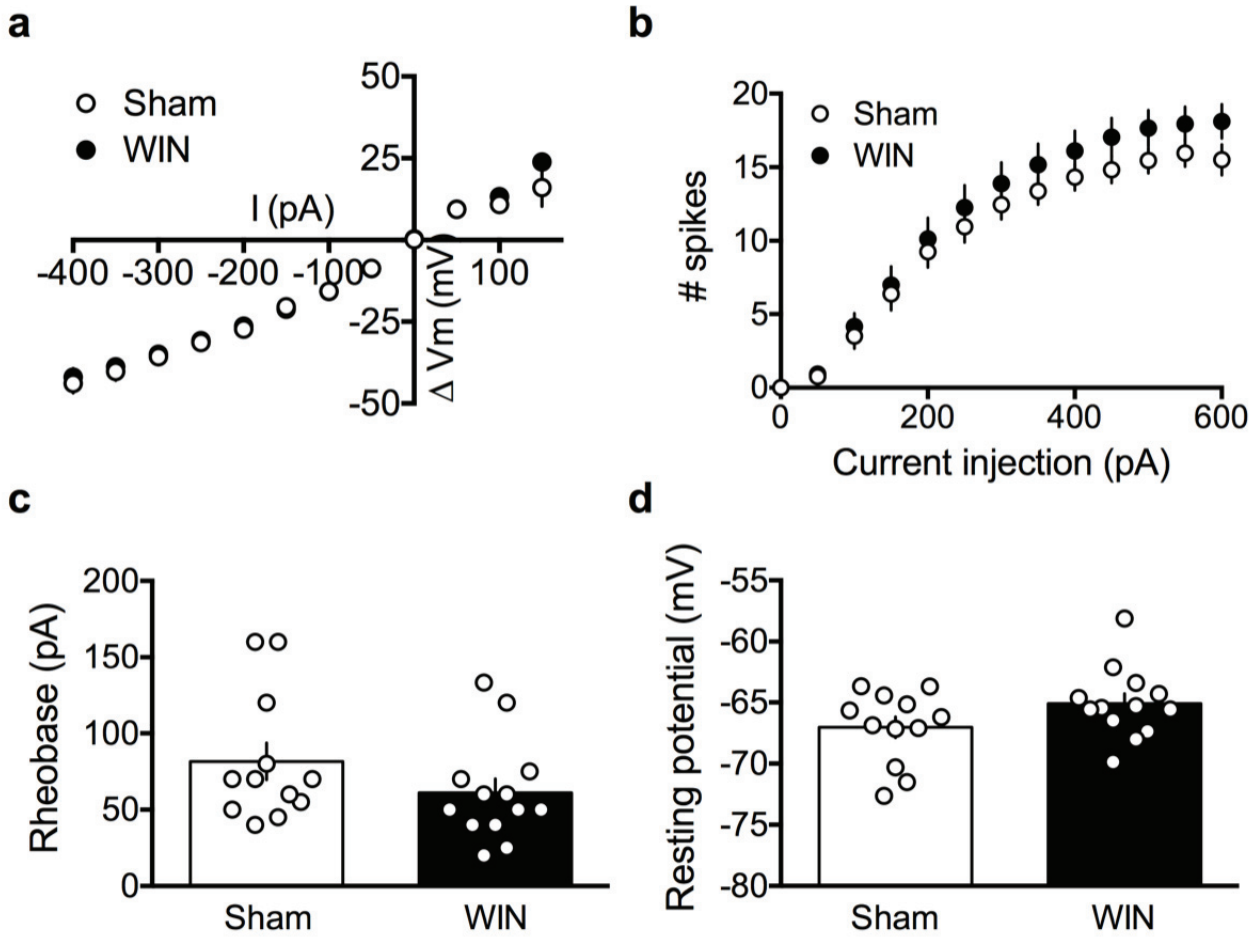
Figure 3. Perinatal WIN exposure does not alter properties of intrinsic excitability of deep layer pyramidal neurons in the PFC of adult offspring.
a: Current injection steps of 50pA from -400pA to 150pA revealed no differences in the I-V relationship in pyramidal neurons of the PFC between the adult offspring of sham- and WIN-treated dams (N = 12, 13 respectively). **b**: Action potentials elicited by progressive current injections from 0-600pA revealed no difference in the number of spikes elicited in pyramidal neurons of the PFC in slices obtained from the adult offspring of WIN-injected dams as compared to those from sham-treated dams (N = 13, 12 respectively; Two-way RM ANOVA, $F_{20,460}$ = 1.112, P = 0.3328). **c**: Progressive current injections in 10pA steps from 0-200pA revealed that the minimum current injection required to elicit an action potential (i.e. rheobase) did not differ in deep layer pyramidal neurons of PFC slices obtained from the adult offspring of WIN- as compared to sham-treated dams (N = 13, 12 respectively; Two-tailed t-test, P = 0.1896). **d**: Similarly, no difference was found in the resting membrane potential of deep layer pyramidal cells in PFC slices obtained from the adult offspring of WIN-treated dams, as compared to those obtained from sham-treated dams (N = 13, 12 respectively; Two-tailed t-test, P = 0.1123). *P<0.05

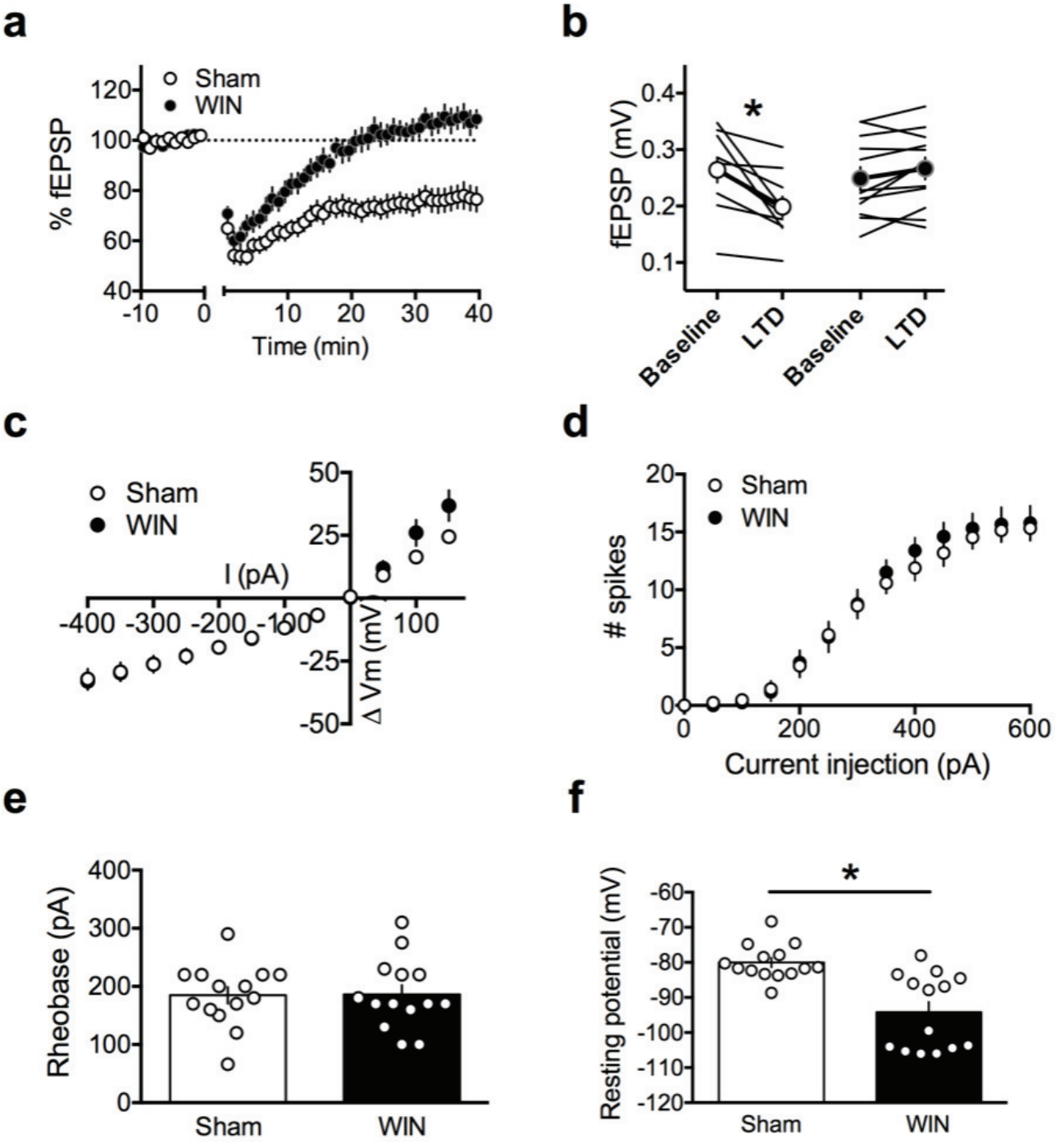
Figure 4. Perinatal WIN exposure abolishes LTD in the NAc of adult offspring and alters the resting membrane potential of NAc medium spiny neurons.
a: A 10-minute, 10Hz local field stimulation of the NAc of the adult offspring of Sham-treated dams (N = 10) elicited a robust eCB-LTD. However, this same protocol failed to induce eCB-LTD in the adult offspring of dams treat with WIN (N = 12). **b**: fEPSP magnitude at baseline (-10 to 0 minutes) and LTD (35-40 minutes post-tetanus) values corresponding to the normalized values in **a** (Two-way RM ANOVA, $F_{1,20}$ = 20.49, P = 0.0002. Sidak's multiple comparisons test, P = 0.0002 and 0.3087 for Sham and WIN, respectively). **c**: Current injection steps of 50pA from -400pA to 150pA revealed no differences in the I-V relationship in medium spiny neurons of the NAc between the adult offspring of sham- and WIN-treated dams (N = 14, 14 respectively). **b**: Action potentials elicited by progressive current injections from 0-600pA revealed no difference in the number of spikes elicited in pyramidal neurons of the PFC in slices obtained from the adult offspring of WIN-injected dams as compared to those from sham-treated dams (N = 14, 14 respectively; Two-way RM ANOVA, $F_{20,250}$ = 0.6092, P = 0.9071). **c**: Progressive current injections in 10pA steps from 0-200pA revealed that the minimum current injection required to elicit an action potential (i.e. rheobase) did not differ in medium spiny neurons of NAc slices obtained from the adult offspring of WIN- as compared to sham-treated dams (N=14, 14 respectively; Two-tailed t-test, P = 0.9502). **d**: However, medium spiny neurons in NAc slices obtained from the adult offspring of WIN-treated dams exhibited significantly lower resting membrane potentials than those obtained from Sham-exposed offspring (N=14, 14 respectively; Two-tailed t-test, P = 0.0003). *P<0.05

Figure 5. Perinatal WIN exposure increases sucrose preference in adult offspring.
a: Total quantities of water and 5% sucrose solution (ml) did not differ between the adult offspring of Sham- or WIN-treated dams during a 20-minute sucrose preference test (N = 8, 8 respectively). **b**: Preference for the 5% sucrose solution over water was significantly higher in WIN- as compared to Sham-treated rats (Two-tailed T-test, P = 0.0091).









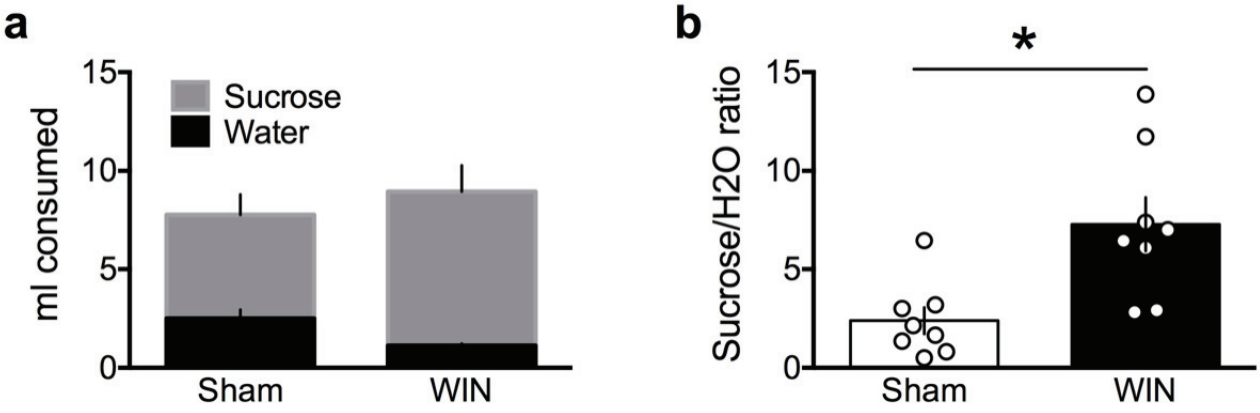


Table 1. Social approach and social memory data by sex

Condition	Test – Measure (unit)	Value	T-test (M v F)
Sham male	Social Preference (ratio)	0.8647 ± 0.01743 N=15	P = 0.5089
Sham female	Social Preference (ratio)	0.8375 ± 0.03569 N=8	
WIN male	Social Preference (ratio)	0.9491 ± 0.01282 N=11	P = 0.0255
WIN female	Social Preference (ratio)	0.8618 ± 0.03219 N=11	
Sham male	Social Memory (ratio)	0.6147 ± 0.03886 N=15	P = 0.0876
Sham female	Social Memory (ratio)	0.7463 ± 0.05970 N=8	
WIN male	Social Memory (ratio)	0.6282 ± 0.05435 N=11	P > 0.9999
WIN female	Social Memory (ratio)	0.6282 ± 0.03590 N=11	
Sham male	Social Preference – Time exploring object (sec)	14.20 ± 1.853 N=15	P = 0.8121
Sham female	Social Preference – Time exploring object (sec)	13.61 ± 1.610 N=8	
WIN male	Social Preference – Time exploring object (sec)	6.586 ± 1.840 N=11	P = 0.0420
WIN female	Social Preference – Time exploring object (sec)	16.05 ± 3.822 N=11	
Sham male	Social Preference – Time exploring rat (sec)	95.38 ± 8.016 N=15	P = 0.3569
Sham female	Social Preference – Time exploring rat (sec)	83.02 ± 10.25 N=8	
WIN male	Social Preference – Time exploring rat (sec)	110.2 ± 9.669 N=11	P = 0.4518
WIN female	Social Preference – Time exploring rat (sec)	100.8 ± 7.422 N=11	
Sham male	Social Memory – Time exploring familiar rat (sec)	29.31 ± 3.733 N=15	P = 0.2200
Sham female	Social Memory – Time exploring familiar rat (sec)	20.90 ± 5.375 N=8	
WIN male	Social Memory – Time exploring familiar rat (sec)	26.17 ± 3.754 N=11	P = 0.3621
WIN female	Social Memory – Time exploring familiar rat (sec)	35.52 ± 2.748 N=11	
Sham male	Social Memory – Time exploring novel rat (sec)	47.06 ± 4.528 N=15	P = 0.1116
Sham female	Social Memory – Time exploring novel rat (sec)	58.58 ± 5.159 N=8	
WIN male	Social Memory – Time exploring novel rat (sec)	49.36 ± 8.196 N=11	P = 0.7014
WIN female	Social Memory – Time exploring novel rat (sec)	53.04 ± 4.657 N=11	

Table 2. Open field data by sex

Condition	Test – Measure (unit)	Value	T-test (M v F)
Sham male	Open Field – Distance (cm)	3120 ± 80.10 N=22	P = 0.1490
Sham female	Open Field – Distance (cm)	3348 ± 135.8 N=17	
WIN male	Open Field – Distance (cm)	2861 ± 151.9 N=14	P = 0.0070
WIN female	Open Field – Distance (cm)	3396 ± 97.26 N=17	
Sham male	Open Field – Rearing (#)	62.23 ± 3.365 N=22	P = 0.2443
Sham female	Open Field – Rearing (#)	68.18 ± 3.735 N=17	
WIN male	Open Field – Rearing (#)	56.64 ± 5.840 N=14	P = 0.0333
WIN female	Open Field – Rearing (#)	71.76 ± 3.118 N=17	
Sham male	Open Field – Center (sec)	54.85 ± 5.348 N=22	P = 0.0839
Sham female	Open Field – Center (sec)	42.24 ± 4.674 N=17	
WIN male	Open Field – Center (sec)	55.87 ± 8.835 N=14	P = 0.0104
WIN female	Open Field – Center (sec)	28.92 ± 2.733 N=17	

Table 3. PFC LTD data by sex

Condition	Test – Measure (unit)	Value	T-test (M v F)
Sham male	eCB-LTD – Normalized fEPSP (35-40min post-tetanus)	80.61 ± 3.408 N=8	P = 0.5478
Sham female	eCB-LTD – Normalized fEPSP (35-40min post-tetanus)	83.90 ± 4.061 N=6	
WIN male	eCB-LTD – Normalized fEPSP (35-40min post-tetanus)	103.9 ± 8.384 N=6	P = 0.7042
WIN female	eCB-LTD – Normalized fEPSP (35-40min post-tetanus)	108.8 ± 9.488 N=7	
Sham male	mGlu2/3-LTD – Normalized fEPSP (35-40min post-drug)	66.26 ± 4.196 N=6	P = 0.8390
Sham female	mGlu2/3-LTD – Normalized fEPSP (35-40min post-drug)	67.58 ± 4.700 N=6	
WIN male	mGlu2/3-LTD – Normalized fEPSP (35-40min post-drug)	67.48 ± 4.156 N=7	P = 0.4467
WIN female	mGlu2/3-LTD – Normalized fEPSP (35-40min post-drug)	72.00 ± 3.900 N=5	

Table 4. PFC LTP data by sex

Condition	Test – Measure (unit)	Value	T-test (M v F)
Sham male	TBS-LTP – Normalized fEPSP (35-40min post-tetanus)	185.6 ± 26.32 N=6	P = 0.3101
Sham female	TBS-LTP – Normalized fEPSP (35-40min post-tetanus)	155.4 ± 6.180 N=7	
WIN male	TBS-LTP – Normalized fEPSP (35-40min post-tetanus)	168.0 ± 11.98 N=9	P = 0.3917
WIN female	TBS-LTP – Normalized fEPSP (35-40min post-tetanus)	152.6 ± 12.58 N=6	

Table 5. NAc LTD data by sex

Condition	Test – Measure (unit)	Value	T-test (M v F)
Sham male	TBS-LTP – Normalized fEPSP (35-40min post-tetanus)	82.37 ± 6.937 N=5	P = 0.2893
Sham female	TBS-LTP – Normalized fEPSP (35-40min post-tetanus)	71.51 ± 6.589 N=5	
WIN male	TBS-LTP – Normalized fEPSP (35-40min post-tetanus)	110.9 ± 6.257 N=5	P = 0.0714
WIN female	TBS-LTP – Normalized fEPSP (35-40min post-tetanus)	95.53 ± 3.039 N=4	

Table 6. PFC intrinsic properties data by sex

Condition	Test – Measure (unit)	Value	T-test (M v F)
Sham male	Resting membrane potential (mV)	-67.95 ± 1.408 N=6	P = 0.3580
Sham female	Resting membrane potential (mV)	-66.11 ± 0.9624 N=6	
WIN male	Resting membrane potential (mV)	-64.12 ± 1.103 N=7	P = 0.2000
WIN female	Resting membrane potential (mV)	-66.22 ± 1.077 N=6	
Sham male	Rheobase (pA)	86,67 ± 18.06 N=6	P = 0.7003
Sham female	Rheobase (pA)	76.67 ± 17.64 N=6	
WIN male	Rheobase (pA)	66.19 ± 16.34 N=7	P = 0.5487
WIN female	Rheobase (pA)	55.00 ± 7.303 N=6	

Table 7. NAc intrinsic properties data by sex

Condition	Test – Measure (unit)	Value	T-test (M v F)
Sham male	Resting membrane potential (mV)	-83.17 ± 1.463 N=5	P = 0.0491
Sham female	Resting membrane potential (mV)	-78.26 ± 1.684 N=9	
WIN male	Resting membrane potential (mV)	-93.95 ± 3.909 N=8	P = 0.5281
WIN female	Resting membrane potential (mV)	-1004 ± 8.945 N=6	
Sham male	Rheobase (pA)	199.2 ± 36.64 N=5	P = 0.5827
Sham female	Rheobase (pA)	176.7 ± 10.93 N=9	
WIN male	Rheobase (pA)	190.6 ± 20.19 N=8	P = 0.7663
WIN female	Rheobase (pA)	180.0 ± 28.28 N=6	

Table 8. Sucrose preference data by sex

Condition	Test – Measure (unit)	Value	T-test (M v F)
Sham male	Water consumed (ml)	1.425 ± 0.1181 N=4	**P = 0.0045**
Sham female	Water consumed (ml)	3.575 ± 0.3326 N=4	
WIN male	Water consumed (ml)	1.075 ± 0.2287 N=4	P = 0.6953
WIN female	Water consumed (ml)	1.175 ± 0.04787 N=4	
Sham male	Sucrose consumed (ml)	3.550 ± 1.533 N=4	P = 0.1069
Sham female	Sucrose consumed (ml)	7.000 ± 0.7153 N=4	
WIN male	Sucrose consumed (ml)	8.800 ± 1.564 N=4	P = 0.5118
WIN female	Sucrose consumed (ml)	6.850 ± 2.291 N=4	
Sham male	Sucrose preference (ratio)	2.739 ± 1.376 N=4	P = 0.6530
Sham female	Sucrose preference (ratio)	2.039 ± 0.3597 N=4	
WIN male	Sucrose preference (ratio)	8.674 ± 1.744 N=4	P = 0.3462
WIN female	Sucrose preference (ratio)	5.885 ± 2.089 N=4	