Inhibition of oxytocin neurons during key periods of development has long-term behavioural and body composition effects

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ABSTRACT

Oxytocin (OT) is a neuromodulator of social behaviour in mammals and accumulative data support the concept of a critical period for OT action during infancy. However, it is possible that the specific functions of OT depend on different time periods of action.

In this study, we aimed to determine whether there are developmental stages during which OT-expressing neurons play a decisive role with long-term consequences. To this end, we chemogenetically inhibited OT-expressing neurons during three critical periods of development (in infants, juveniles, and young adults) in male and female mice, followed by a longitudinal study to assess behaviour and metabolic perturbations. The most pronounced behavioural effects are observed after inhibition during infancy in both sexes. Notably, social memory is consistently impaired in males, regardless of the inactivation period. From a metabolic perspective, in adulthood, an increase in body weight is observed in all cohorts of males but fat mass and adipocyte size increase after inhibition of OT-expressing neurons during juvenile period. In addition, we observed a significant delay in the day of birth and an alteration in feeding behaviour in neonates after inhibiting OT-expressing neurons around the time of birth.

Thus, inhibition of OT neurons during three postnatal periods has direct, distinct, and long-lasting consequences on social behaviour and metabolism, depending on the sex and on the timing of inactivation. Our results also demonstrate a role of foetal/neonate oxytocin neurons in the timing of birth and in early feeding behaviour.

INTRODUCTION

Oxytocin (OT) is involved in many physiological processes and is a powerful neuromodulator of social behaviour in primates and rodents[1,2]. Over the last two decades, studies have focused on the role of OT in neurodevelopment and suggested a critical role for OT during the early postnatal developmental period[3,4]. In particular, OT manipulations after birth are well-documented in prairie voles, producing effects that depend on sex, dose, and region[5,6].

OT has been implicated in the pathophysiology of several neurodevelopmental disorders, including Autism Spectrum Disorders (ASD), Prader-Willi syndrome (PWS) and Schaaf Yang syndrome[7,8], as well as postnatal stress and anxiety[9] and in post-traumatic syndrome disorder (PTSD)[10]. OT treatments were administered to patients with ASD and PWS[11], stress-related psychiatric disorders or PTSD[12]. Although positive therapeutic effects were observed, there was discrepancy in the results, probably because the patient cohorts are heterogeneous in terms of age, cause and environment and the administration methods, dose and duration were not based on sound scientific basis [11,12,13,14]. However, OT-treatment in infants[15,16] or children with PWS[17,18,19,20] has a beneficial and long-term impact on feeding and social behaviour[16].

In several rodent models with knock-out (KO) of ASD risk genes *(Magel2, Cntnap2, Fmr1, Nlgn3, Shank3, Oprm1, Pten*) or in BTBR mice and valproic acid exposed rats or mice, OT system has been implicated in social deficits[21]. Furthermore, OT appears as a convergent signaling pathway in autism[22, 23]**.** Importantly, OT treatment during infancy has been shown to restore normal social behaviour in most of these models, with effects lasting into adulthood, as demonstrated in Magel2 KO[24,25], Cntnap2 KO[26]; Fmr1 KO[27]; the 22q11.2 hemideletion syndrome mouse model[28], alproic acid treated mice[29] and also in a rat model of PTSD[30].

Altogether, these results support the concept of an “hormonal imprint” of OT early in life, which shapes the brain circuits involved in social behaviour in the long term, thus defining a critical period of action during infancy. A recent study suggests that the early postnatal period may be the only critical period during which oxytocin promotes prosocial behaviors by regulating the GABA shift during postnatal brain development[29]. It is also conceivable that there are different

critical periods of development during which OT triggers specific behaviours or metabolic responses. Indeed, social conditioned place preference in mouse is influenced by OT action within a window of time from weaning to early adulthood but not infancy[31].

Despite these data, a concomittent study to assess the direct consequence of an OT during restricted periods of development, has never been studied. Such a study would be important for understanding the pathogenesis of neurodevelopmental diseases involving the OT system and for developing appropriate therapeutic approaches that target or reopen those periods[31]. Therefore, in order to establish a causal link between the lack of OT neuronal activity (and OT release) during a specific stage and the altered phenotype, it is important to assess the direct impact of inhibiting OT neuronal activity at certain periods of development. In this study, we used chemogenetics to inhibit OT-expressing neurons at three stages of development: the first week of life, juvenile (puberty) male or female, and young adulthood (P60). We specifically assessed behavioural and metabolic consequences. To evaluate the importance of OT produced by newborns, OT-expressing neurons were inactivated from the 18th day of gestation until birth. These time points were chosen because they span important milestones in brain development, such as the maturation and connectivity of OT-expressing neurons[32] and the dynamic expression of OT receptors[33].

RESULTS

Chemogenetic inhibition of OT-expressing neurons at three postnatal stages.

To inhibit OT neurons temporarily, we generated a transgenic mouse model (OT-Cre::hM4Di-Ha), which expresses an inhibitory $G_{\alpha i/o}$-protein coupled receptor (hM4Di, R26-LSL-hM4Di-Ha) from the Designer Receptors Exclusively Activated by Designer Drugs (DREADD) system in OT-expressing neurons. Adapted crossings were performed to obtain OT-Cre heterozygous::hM4Di-ha homozygous (named Cre+) and hM4Di-Ha homozygous (named Cre-) mice in each litter (Fig. 1a). We validated the expression of the hM4Di-Ha receptor in OT-expressing neurons only (Fig. 1b). Using electrophysiology on brain slices, including the

PVH of five-day-old mice (P5), we performed whole-cell current-clamp recordings and confirmed that the DREADD ligand Compound 21 (C21) efficiently inhibited the action potentials of OT-expressing neurons (Fig. 1c) in Cre+ mice only. We chose C21 because it is a potent and selective agonist for hM4Di, with a duration of action of six hours, and excellent bioavailability and brain penetrability[34,35]. Furthermore, C21 exhibits weaker off-target responses across a broad spectrum of behavioural measures compared to controls, even with chronic administration over several weeks[35,36]. Nevertheless, mice lacking hM4Di (Cre-) injected with C21 were always included as the control group. Importantly, we also validated the absence of long-term alterations to OT neuron survival, following treatment with C21 at different ages and Cre expression in OT neurons, in all the studied cohorts. We found that male and female Cre+ and Cre- mice, treated with C21 at 5 months of age, did not display alterations in the number of OT+ neurons in the PVH[37].

OT neuron's inhibition was achieved by administering C21 (2.5 mg/kg; s.c. or i.p. injections) twice daily (at 9 am and 5 pm) to cover the active period. Male and female mice were treated during three developmental periods: infancy (P0–P7), puberty (P22–P28 for females) and (P28-P34 for males), and young adult (P56-P62) (Fig.1d).

The following tests were performed: rotarod test was performed on all cohorts of mice at 2.5 months to evaluate their sensory motor abilities. Between 2.5 and 3.5 months of age, spontaneous behaviour, exploration of the environment, social interactions and anxiety were assessed in an open field (OF). The elevated plus maze (EPM) test was performed to specifically assess anxiety. We also used the novel object recognition (NOR) test to investigate learning and memory in mice. Finally, the three-chamber test was used to assess social exploration (sociability), preference for social novelty (social discrimination) and short-term social memory. This test was only performed on four-month-old males, as it is not adapted for females[25] (Fig. 1d). We also assess the effect of OT-inhibition on body weight, fat mass, and adipocytes.

Chemogenetic inhibition of OT-expressing neurons in neonates impairs ultrasonic vocalizations (USVs) in infancy and behaviour at adulthood.

We generated a specific cohort to assess ultrasonic vocalisations (USVs cohort) at P8, after chemogenetic inhibition of OT-expressing neurons during the first week of life (Fig. 1d). We observed a decrease in the number of USVs in Cre+ pups compared with Cre- pups (Fig. 2b), regardless of sex. A deficit in USVs in pups (at P8) separated from their mothers reveals an alteration in communication between the pup and its mother[38,39]. We generated a separate cohort (Fig. 2a), in which we performed all tests in adulthood. The rotarod test (Fig. 2c) revealed no significant differences between Cre+ and Cre- males or females over three days of testing, except for a slight increase in activity in Cre+ females on the first day. In the open field test (Fig. 2d), male and female control mice (Cre-) spent more time exploring the periphery (Per) than the centre (Cen) of the field. However, male and female Cre+ mice spent the same amount of time in both spaces, suggesting a reduction in anxious behaviour or a deficit in recognising the explored environment. Locomotor activity (distance covered in the OF) is similar in both populations and sexes (Fig. 2e), and in the EPM test, all mice exhibit similar behaviour (Supplementary Fig. 1a), indicating normal anxiety levels. Cre+ males tend to spend more time self-grooming than Cre- males, while Cre+ females spend less time self-grooming than Cre- females (Fig. 2f). Self-grooming is considered a measure of repetitive behaviour and involves the PVH[40]. Rearing frequency is similar in all groups (Fig. 2g). In the NOR test, both male and female Cre- mice prefer to explore the novel object, whereas Cre+ mice spend the same amount of time exploring both objects (Fig. 2h). Total exploration time is similar in all groups (Fig. 2i). Cre+ mice do not appear to be interested in novelty or recognise a familiar object from a novel one. Spontaneous social interactions (SSI) were similar between Cre+ and Cre- males and between Cre+ and Cre- females (Fig. 2j). In a three-chamber test, both Cre+ and Cre- mice interacted significantly more with a mouse than with an empty grid. However, in the social novelty paradigm, Cre+ males did not show a preference for the novel mouse (social novelty), and a social memory deficit was observed in these males (Fig. 2k).

Taken together, these data demonstrate that inhibiting OT-expressing neurons during the first week of postnatal life impairs the number of USVs in male and female pups. Long-lasting effects are observed in both sexes in terms of exploration and recognition of a novel environment (centre versus periphery in OF and novel versus familiar object in NOR), but Cre+ mice do not exhibit increased anxiety. Cre+ mice interact socially with conspecifics, as demonstrated by the SSI and socialisation tests performed in the three-chamber test. However, preference for social novelty and social memory are impaired in males. This could be due to a lack of recognition or interest in novelty and a social memory deficit. Additionally, differences in self-grooming activity are observed between Cre+ and Cre- males or females.

Alterations in social behaviour are often associated with an imbalance between excitatory and inhibitory (E:I) activity in the hippocampus, as demonstrated in a mouse model of autism involving a neonatal oxytocin deficiency[25]. An oxytocin neonatal treatment was shown to improve this E:I imbalance[25]. Using whole-cell recordings in acute brain slices, we examined the spontaneous activity of hippocampal CA3 pyramidal neurons in juvenile Cre+ and Cre- mice and did not observe an E:I imbalance (Supplementary Fig. 2).

Chemogenetic inhibition of OT-expressing neurons during male puberty impairs social behaviour at adulhood.

Male and female mice were treated with C21 during puberty period (Fig. 3a). We took in consideration the fact that puberty in male and female mice do not occur during the same period, puberty in males starting 4 days later[41]. We specifically treated the mice across the pubertal transition when hypothalamic genes and *Oxt* gene peak expression (P27 in females and P32 in males)[42]. At adulthood, no sensory-motor deficit (rotarod test) was observed between the males or females Cre+ and Cre- (Fig. 3b). In the OF, only Cre+ males spend the same quantity of time in periphery and in the center, all other groups of mice (Cre- males, Cre+ and Cre- females) spend more time in periphery, as expected (Fig. 3c). However, all mice cover similar distance in OF (Fig. 3d) and in EPM test no difference is observed between the groups of mice (Supplemental Fig. 1b), suggesting that Cre+ males have a deficit in

exploration/ recognition of environment. Grooming duration (Fig. 3e) and rearing (Fig. 3f) are not altered. In the NOR test, all mice interact more time with the novel object (Fig. 3f) but the time for each exploration (Fig. 3f) and total exploration time are reduced in Cre+ males (Fig. 3g) with both the novel or same object (Fig. 3h). Spontaneous social interactions were similar in Cre+ and Cre- males and in Cre+ and Cre- females (Fig. 3i). In the 3 chamber test, Cre+ males display a deficit in social memory and even if they prefer to interact with the novel mouse in social novelty, their preference for novelty is clearly weaker compared with Cre- males (Fig. 3j).

Taken together, these data show that inhibiting OT-expressing neurons during female puberty has no effect on behavioural tests performed in adulthood. However, inhibiting OT-expressing neurons during male puberty impairs exploration and recognition of a novel environment (centre versus periphery and exploration time with objects), though mice do not become more anxious. In addition, social memory is impaired in the 3-chamber test. As suggested above, the alterations observed in Cre+ males may be due to a lack of recognition of novelty and/or memory deficits, or a lack of interest in novelty, similar to the effects observed following inhibition during the first week of life.

Chemogenetic inhibition of OT-expressing neurons in male young adults impairs social behaviour at adulhood.

Both male and female mice were treated with C21 between P56 and P62 (Fig. 4A). The rotarod, EPM, spontaneous behaviour in the open field (OF) and social-social interaction (SSI) tests were performed on 2.5–3.5-month-old mice, and the three-chamber test was performed on 4–4.5-month-old males. In adulthood, no sensory-motor deficits were revealed in the rotarod test between males or females Cre+ and Cre- (Fig. 4b). In the open field test, all groups of mice spent more time in the periphery (Fig. 4c). In the OF test, only Cre+ males showed a deficit in distance travelled (Fig. 4d) and in the number of rearings (Fig. 4f), suggesting a slight reduction in activity. No alteration is observed in the NOR test in Cre+ mice (Fig. 4g–h). Cre+

males show an increase in the duration of spontaneous interactions with another mouse (Fig. 4i). In the three-chamber test, Cre+ males exhibited a deficit in social memory (Fig. 4j).

Taken together, these data demonstrate that inhibiting OT-expressing neurons in young adult males (two months old) also has long-lasting effects, with a slight reduction in activity in the open field and a deficit in social memory.

Consequences of chemogenetic inhibition of OT-expressing neurons during infancy, juvenile and in young adults on body weight and body composition.

Oxytocin is well known for its anorexigenic role in food intake and energy balance. Therefore, it is important to evaluate the metabolic consequences of chemogenetically inhibiting OT-expressing neurons during the infant, juvenile, and adult stages. Firstly, we compared the weight of different cohorts of three-month-old male and female mice with inhibited OT-expressing neurons (Fig. 5a). We observed an increase in weight in all Cre+ males following inhibition of OT-expressing neurons in infancy (+9%), in the juvenile period (+17%) or in young adulthood (+19%) (Fig. 5a). An increase in fat mass (perigonadal adipose tissue, +82%; Fig. 5b) and adipocyte size (perigonadal adipose tissue, +62%; Fig. 5c, g–h) is also associated with OT neuron inhibition in juvenile males only, but not when inhibition occurred in neonatal life or young adults. All cohorts of Cre+ females have a similar weight (Fig. 5d), fat mass (Fig. 5e) and adipocyte size (Fig. 5f, g–h).

Thus, inhibiting OT-expressing neurons during male puberty only, increases weight, fat mass, and adipocyte size. Inhibition in infancy or in young adult males, increases weight at P100, but has no effect on fat mass or adipocyte size.

Inhibition of OT-expressing neurons in foetuses from gestational day 18 to the day of birth delays the delivery day and impairs feeding of neonates

Previous studies have suggested that there is an important role for OT in neonatal development that is caused by the OT produced by the mother just before birth[43], as well as a role for OT produced by the neonate from birth[44,45]. To assess the role of OT produced by

newborns around birth, we inhibited OT-expressing neurons from gestational day 18 (GD18) to birth (P0) by adding 0.25 mg/ml C21 to the drinking water of the mother (the mother is wild-type (Cre-) and carries Cre+ and Cre- foetuses since the OT-Cre is transmitted by the father). This was done on the assumption that C21 could act on foetuses (Fig. 6a). In a litter containing both Cre+ and Cre- pups, we verified that the inhibition of OT neurons, as indicated by cFos immunostaining, occurred in the Cre+ pups, but not in the Cre- pups (Fig. 6b). Unexpectedly, C21 exposure caused a high percentage of delayed deliveries by one or two days when mothers carried Cre+ foetuses, but not when dams only had Cre- foetuses (Fig. 6c). Remarkably, the frequency of delayed deliveries correlated with the number of Cre+ pups in the litters (Fig. 6d). These data suggest that foetal OT-expressing cells participate to the timing of delivery. We hypothesized that the fœtal part of placenta might express fœtal OT. However, at E18.5, we did not detect any fœtal OT expression in the placenta (Supplementary Fig. 3)

We also examined the feeding state of the Cre+ and Cre- pups 12-24 hours after birth, which could be observed by the presence of milk in the stomach. We found that 35% of pups died without milk in their stomachs, while 53% had only a small quantity of milk and only 10% had a high quantity of milk. This finding was markedly different in the Cre- pups, with no mortality and 60% having a high quantity of milk in their stomachs. This suggests that inhibiting OT-expressing neurons in neonates alters suckling.

DISCUSSION

The aim of this study was to clarify whether there are developmental stages during which OT-expressing neurons have long-term behavioural consequences. To this aim, we conducted a concomitant study involving the chemogenetic inhibition of OT-expressing neurons during three critical developmental periods (in infants, juveniles and young adults) followed by a longitudinal study to evaluate behavioural outcomes and metabolic issues in male and female mice. Regardless of the stage of inhibition, motor ability and anxiety levels remained unaffected. However, specific deficits in social behaviour were observed depending on the period of inhibition and sex (see Table 1). The strongest behavioural effects impact both sexes

and were observed following chemogenetic inhibition during the first week of postnatal life. Notably, social memory is consistently impaired in males regardless of the period of inactivation. Metabolically, an impact on body weight was observed in males only whatever the stage of inhibition, but fat mass and adipocyte average size in adulthood were increased following inhibition of OT-expressing neurons during the juvenile period only. In addition, we observed a significant delay in the day of birth in neonates after we inhibited OT-expressing neurons around the time of birth. Furthermore, we noted a deficit in feeding behaviour in the neonates.

Inhibition during the first week of life in male and female newborns leads to a more severe phenotype than inhibition at later stages. We demonstrate a reduced number of ultrasonic vocalisations (USVs), and a deficit in recognizing the explored environment and a lack of interest in novelty or difficulty distinguishing between familiar and novel objects. Additionally, in a three-chamber test, males exhibit good interactions with congeners, but do not show preferences for novel versus familiar mice. They also demonstrate deficits in social memory. Our data are consistent with the phenotypes observed in constitutive OT-KO [29, 39,46], OT-receptor-KO mice[38,47,48], and CD-38 knockout (CD-38 being necessary for oxytocin release) mice[49,50] and strongly suggest that the phenotype of those transgenic mice results from developmental alterations. Furthermore, it has been demonstrated that OT treatment in mouse models of ASD during infancy can rescue normal behaviour in adulthood (see introduction), particularly social memory[25,27]. These long-lasting OT effects could result from the normalization of key developmental hippocampal processes[25,27,30], such as those described in *Magel2 KO* mice, with the restoration of the developmental GABA shift, the excitatory/inhibitory balance, the number of somatostatin-expressing neurons[25] and the normalization of OT receptor expression[51]. This period coincides with the maturation and shaping of OT-related neuronal circuitry. Thus, our results provide evidence of a critical period during the first week of life when OT-expressing neurons are necessary to encode lifelong social behaviours. Interestingly, inhibition of OT-expressing neurons in the first week of life

impacts both sexes (except for social memory, which was tested only on males). During this period, there is no sexual dimorphism in the control of OT-expressing neurons but, after the first week of life, in males only, testosterone induces a sexual dimorphism[52] in OT release, which could explain why sexual dimorphism in behaviour is observed after the inhibition of OT-expressing neurons after infancy period.

Inhibition during the male or female adolescence, triggers deficits in males only (lack of recognition of environment, reduction of time exploring the objects, social memory deficit). Such effects might be conceivable because there is sexual dimorphism and maturation of the hypothalamus function during puberty. Finally, inhibition in young adults alters adult males only, with a loss of social memory, an increase in the duration of spontaneous social interactions and a decrease in the travelled distance and number of rearings measured in the open field. This result is quite unexpected since the OT-system is mature at this age and we do not have yet any mechanistic explanations of how transient inhibition of OT-neurons, in young adults, has a long-term impact on the behavioural phenotype. We suspect epigenetic modifications conducting to alterations in OT-receptor expression that should be investigated.

An increase in body weight, fat mass and adipocyte average size is observed following an inhibition of OT-expressing neurons during the juvenile period in males. But inhibiting OT-expressing neurons during infancy or in young adult males increases body weight without any effects on fat mass and adipocyte size. Together, these results indicate that OT inhibition exerts metabolic effects from infancy until adulthood modulating the body weight but influences adiposity during juvenile period through mechanisms that remain to be investigated.

In addition, to influence metabolism, our recent study[37] showed that OT plays a role in the development of hypothalamic melanocortin neuronal circuits. Chemogenetic inhibition of oxytocin neurons during the first postnatal week disrupts the development of pro-opiomelanocortin and agouti-related peptide neuronal projections specifically to the paraventricular nucleus of the hypothalamus. We also found that this neurodevelopmental

effect of OT neurons is restricted to a neonatal critical period and depends on SNARE-mediated exocytosis from oxytocin neurons.

By administrating C21 in the drinking water of the mother at GD 18.5 and until the day of birth we could inhibit OT-expressing neurons in the Cre+ foetuses and observed a delay in the timing of birth in mothers with both Cre+ and Cre- foetuses but not in those with only Cre- foetuses (controls). These data suggest that inactivation of OT-expressing neurons in Cre+ foetuses has consequences on the timing of delivery/birth. OT is a well-known stimulant of myometrial contraction and exhibits maximum at time of delivery. Liggins[53] showed that, in ewe, the destruction of foetal hypothalamic PVH results in a delayed parturition. Then, it was shown that foetal OT is involved in human and ewe labor[54-56]. More recently[57], it was proposed that the balance between foetal oxytocin and placental leucine aminopeptidase (P-LAP which degradates OT) controls labor onset human via uterine contraction. Thus OT might be one of the foetal-to-maternal signaling in the timing of birth. However, no similar study was conducted in mouse and mature oxytocin seems to be produced from birth and not during foetal development[44,58]. In addition, we did not detect any expression of foetal OT in the placenta of E18.5 foetuses whereas an OT expression was detected in their brain; however further studies need to be done in mouse to clarify the presence of foetal OT in the placenta just before birth, during labor.

In addition, 35% of small Cre+ mice die on the day they are born because they do not feed, and 59% have little or no milk in their stomachs, suggesting that the inactivation of OT-expressing neurons in newborns prevents them from feeding. This result confirms our previous results suggesting a direct or indirect role of OT to trigger suckling in mouse neonates[45].

In conclusion, we have identified different developmental periods during which inhibition of OT-expressing neurons has direct long-term consequences on social behavior and metabolism, depending on the sex of the mouse and the timing of inactivation. Our results also demonstrate the involvement of foetal and neonatal OT-expressing neurons in the timing of birth and early feeding behaviour. These findings clarify the role of these neurons in neurodevelopmental

disorders involving the OT system and could inform the development of therapeutic approaches targeting these periods.

Limitations

OT-expressing neurons produce other neurotransmitters (glutamate) and neuropeptides and also nitric oxide; all can be involved in the phenotypes observed following OT-neurons inactivation. However, combining those results with all the data in the literature (see introduction) showing firstly, that OT is involved in social deficits (in particular social memory) and, secondly, that OT-treatment rescues, with long term effects, these alterations observed in mouse models of ASD; it is highly likely that OT-deficiency is responsible for the observed phenotypes in our study. A similar study with transient inactivation of the OT gene at different developmental periods would confirm our results. This study is not exhaustive and other behavioural and metabolic tests might be performed.

MATERIALS ET METHODS

Study approval

All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (N.R.C., 1996) and the European Communities Council Directive of September 22th 2010 (2010/63/EU, 74) and the approved protocol (APAFIS#21251-2019092314503551 for the studies performed in Marseille and APAFIS# 13387–2017122712209790 for the studies performed in Lille) by the Ethical Committee of the French Ministry of Agriculture. Animals were group-housed with a 12-h light-dark cycle (light from 7pm to 7 am and dark from 7am to 7pm), at a constant temperature (22 ± 1°C) with *ad libitum* access to standard chow and water.

Sex as a biological variable

Our study examined male and female animals. Both sexes were pooled when no sex differences were found.

Mouse strains

Species and genetic background	Designation	Reference	Additionnal informations
Mice, C57BL6/J	WT		Charles River
Mice, C57BL6/J	OT::Cre	(Wu *et al.*) [59]	JAX stock #024234
Mice, C57BL6/J	Ai14(LSL-tdTomato)	(Madisen *et al.*) [60]	JAX Stock 007914
Mice, C57BL6/J	R26-LSL-hM4Di-DREADD	(Zhu *et al.*)[61]	JAX stock #026219

The R26-LSL-hM4Di-DREADD mice were on a C57Bl6/N genetic background, which is not suitable for conducting behavioural analyses, due to a mutation in the gene encoding Cytoplasmic FMR interacting protein 2, which regulates behaviour[62]. We performed over 8 back crosses with C57BL6/J mice and validated by sequencing the deletion of the Cyfip2 mutation in mice used in the breedings to generate all the mice studied.

Chemogenetic inactivation by intra-peritoneal or oral administration of Compound 21

Chemogenetic experiments were performed on OT::Cre-/+;R26-LSL-hM4Di-DREADD/ R26-LSL-hM4Di-DREADD (named Cre+) male and female mice. A subcutaneaous (in infancy) or intraperitoneal (at adolescence or adulthood) injection of C21 (DREADD agonist 21 dihydrochloride, Hello Bio, Cat. No. HB HB6124 Hello Bio Ltd, Ireland) at 2.5mg/kg (diluted in NaCl 0.9%) was performed twice a day (one at 9am and one at 5pm). To inactivate OT-expressing neuronsin late embryos and newborns, we added C21(0.25 mg/mL) with 5mM saccharin (Sigma Aldrich, Cat. No. 109185) in drinking water of the female from Gestational Day 18 to one day after birth. Gestational female at this stage have a body weight around 40g and consume ~5 ml of water per day[63]. Accordingly, C21 dissolved in drinking water at 0.25mg/ml results in 4-6mg/kg of C21 active dose at any time since a mouse drinks all over the 24 hours and the duration of action of a single dose of C21 is around 4-6hours[64]. Mouse

cage water containing C21 and saccharin was carefully monitored and changed for fresh solution every day.

Immunohistochemistery

P20 mice were deeply anaesthetized with intraperitoneal injection of the ketamine/xylazine mixture and transcardially perfused with 0.9% NaCl saline followed by Antigenfix (Diapath, cat #P0014). Brains were post-fixed in Antigenfix overnight at 4°C and included in agar 4%. 50 µm-thick coronal sections were sliced using a vibratom (Zeiss) and stored in PBS at 4°C. Floating slices (of the hippocampal region corresponding to slices 68 to 78 on Allan Brain Atlas) were incubated for 1 hour with blocking solution containing 0.1% (v/v) Triton X-100, 10% (v/v) normal goat serum (NGS) in PBS, at room temperature. Sections were then incubated with primary antibodies diluted in incubation solution (0.1% (v/v) Triton X-100, 3% (v/v) NGS, in PBS), overnight at 4C°. After 3 x 10 min washes in PBS, brain sections were incubated with secondary antibodies diluted in the incubation solution, for 2 hours at RT. Sections were washed 3 x 10 min in PBS and mounted in Flouromount-G (EMS, cat #17984-25).

Designation	Supplier	Identifiers	Dilution
Mouse polyclonal anti-oxytocin (prohormone)	A gift of H. Gainer	PS38	1/50
Rabbit polyclonal anti-cFos	Abcam	AB190289	1/1000
Rabbit polyclonal anti-HA tag	Cell Signaling Technology	2367S	1/300
Alexa 488-conjugated donkey polyclonal anti-rabbit	Invitrogen	A21206	1/500
Alexa 647-conjugated donkey polyclonal anti-mouse	Invitrogen	A-31573	1/500

Images were acquired using a fluorescence microscope (Zeiss Axioplan 2 microscope with an Apotome module).

Electrophysiological experiments

PVH slice preparations and recording of OT neurons

OT-Cre::Ai14(LSL-tdTomato)::R26-LSL-hM4Di-DREADD mice at P5 were anesthetized using 5% isoflurane and rapidly decapitated. Coronal slices containing the PVH (350 µm) were generated using a vibratome (VT 1200S, Leica, Germany). Slices were allowed to equilibrate before use for 1h in standard recording aCSF (in mM: 124 NaCl, 3 KCl, 1.2 NaH2PO4, 1.2 MgSO4, 25 NaHCO3, 11 D-glucose, and 2 CaCl2, saturated with 95% O2/5% CO2, pH 7.4, ~300 mosM) at room temperature. During the experiments the recording chamber was superfused at ~3 mL/min with the standard recording aCSF.

Electrodes (resistance ~3.0-6.0 MOhm) were prepared using a Narishige micropipette puller (PC-100) and filled with (in mM) 130 K^+-gluconate, 10 NaCl, 11 EGTA, 1 CaCl2, 10 HEPES, 1 MgCl2, 2 Mg-ATP, 0.2 Na-GTP, pH 7.3, and ~280 mosM. Recording pipettes were guided using a piezoelectric manipulator (Luigs & Neumann, GmbH). $PVH^{OT\text{-}expressing\ neurons}$expressing tdTomato were identified in Cre^+ mice using a Hamamatsu scientific camera. In Cre^- mice, unlabeled neurons in caudal PVH slices were recorded. Following initial membrane rupture the resting membrane potential was measured. Resting membrane potential, spontaneous action potentials, and evoked action potentials were recorded in current clamp mode. The DREADD agonist C21 (10 µM) was applied for 5 min. Data obtained from PVH slice experiments were acquired in pClamp 10.7 using a HEKA EPC 10 Double amplifier (HEKA Elektronik), filtered at 2 kHz and sampled at 20 kHz. Data were analyzed using Clampfit 10.7 and MiniAnalysis (Synaptosoft, USA).

Hippocampal slice preparation and recordings of pyramidal CA3 neurons

Brains were removed and immersed into ice-cold (2-4°C) artificial cerebrospinal fluid (ACSF) with the following composition (in mM): 126 NaCl, 3.5 KCl, 2 $CaCl_2$, 1.3 $MgCl_2$, 1.2 NaH_2PO_4, 25 $NaHCO_3$ and 11 glucose, pH 7.4 equilibrated with 95% O_2 and 5% CO_2. Hippocampal slices (400 µm thick) were cut with a vibratome (Leica VT 1000s, Germany) in ice cold oxygenated choline-replaced ACSF and were allowed to recover at least 90 min in ACSF at room (25°C)

temperature. Slices were then transferred to a submerged recording chamber perfused with oxygenated (95% O_2 and 5% CO_2) ACSF (3 ml/min) at 34°C.

Whole-cell patch clamp recordings were performed from P20-P25 CA3 pyramidal neurons in voltage-clamp mode using an Axopatch 200B (Axon Instrument, USA). To record the spontaneous and miniature synaptic activity, the glass recording electrodes (4-7 MΩ) were filled with a solution containing (in mM): 100 KGluconate, 13 KCl, 10 HEPES, 1.1 EGTA, 0.1 $CaCl_2$, 4 MgATP and 0.3 NaGTP. The pH of the intracellular solution was adjusted to 7.2 and the osmolality to 280 mOsmol l^{-1}. The access resistance ranged between 15 to 30 MΩ. With this solution, the $GABA_A$ receptor-mediated postsynaptic current (GABAA-PSCs) reversed at -70mV. GABA-PSCs and glutamate mediated synaptic current (Glut-PSCs) were recorded at a holding potential of -45mV. At this potential GABA-PSC are outwards and Glut-PSCs are inwards. Spontaneous synaptic activity was recorded in control ACSF and miniature synaptic activity was recorded in ACSF supplemented with tetrodotoxin (TTX, 1µM). Spontaneous and miniature GABA-PSCs and Glut-PSCs were recorded with Axoscope software version 8.1 (Axon Instruments) and analyzed offline with Mini Analysis Program version 6.0 (Synaptosoft).

Behavioural studies

Ultrasonic Vocalisations

Early social communication was tested by analyzing isolation-induced ultrasonic vocalizations (USVs) in P8 pups of both sexes[65]. Briefly, the mother and litter were left together for 30 min to habituate to the testing room, then P8 pups were all isolated from the mother and gently transferred to a new cage on a heating pad (37°C). After 5 min of separation, each pup was transferred in an isolation box located inside an anechoic box (54 x 57 x 41 cm; Couldbourn instruments, PA, USA). USVs were recorded for 300 s by an ultrasonic microphone (Avisoft UltraSoundGate condenser microphone capsule CM16/CMPA, Avisoft bioacustic, Germany) sensitive to frequencies of 10-250 kHz. Recordings were done using Avisoft recorder software (version 4.2) with a sampling rate of 250 kHz in 16-bit format. Data were analyzed for the total number of calls using Avisoft SASLab software.

Rotarod. Mice were challenged to walk on a rotating rod that increases in speed over a predetermined period of time (for 5 min) until the animal falls. The latency to fall from the rod is determined and taken as a measure of motor function. The test is performed over three successive days. The daily trial consists of three measures spaced by 5 minutes, a mean of the three measures is considered for each day.

Elevated plus maze. The device consists of a labyrinth of 4 arms of 5 cm width located 80 cm above the ground. Two opposite arms are open (without wall) while the other two arms are closed by side walls. The light intensity was adjusted to 20 Lux on the open arms. Mice were initially placed on the central platform and left free to explore the cross-shaped labyrinth for 5 minutes. The maze was cleaned and wiped with H_2O and with 70% ethanol between each mouse. Animal movement was video-tracked using Ethovision software 11.5 (Noldus). Time spent in open and closed arms, the number of entries in open arms, as well as the distance covered, are directly measured by the software.

Open field. The open field test was performed in a 40 x 40 cm square arena with an indirect illumination of 60 lux. Mouse movement was video-tracked using Ethovision software 11.5 (Noldus) for 10 minutes. Total distance traveled and time in center (exclusion of a 5 cm border arena) are directly measured by the software. Grooming (time and events) and rearing were manually counted in live using manual functions of the software, by an experimented behaviourist. The open-field arena was cleaned and wiped with H_2O and with 70% ethanol between each mouse.

Spontaneous Social Interaction in an open field arena. Animals were first habituated to the apparatus for 30 min. Social interaction was measured using pairs of mice from different housing cages, and having the same genotype, the same gender, and approximately the same body weight. Each pair was placed in the open field arena for 15 min during which different behavioural parameters reflecting social interaction between the two mice were recorded (following and sniffing). A social interaction index was defined as the percentage of counts and duration of social events over the total social and individual events.

New object recognition. First, two identical objects are placed in the arena, and then one of these objects is replaced by a novel object. Normal mice are expected to spend more time exploring the novel object than the familiar one. The arena used for the novel object recognition test was the same used for the open-field test. The arena was cleaned and wiped with 70% ethanol between each mouse. Two identical objects (50 ml orange corning tube) were placed in the opposite corners of the arena, 10 cm from the side walls. The tested mouse was placed at the opposite side of the arena and allowed to explore the arena for 10 min. After 1h, one object was randomly replaced with another novel object, which was of similar size but differ in the shape and color with the previous object (white and blue lego bricks), the other object (same object) was kept. Then, the same mouse was placed in the arena and allowed to explore the two objects (a new and an "old" familiar object) for 10 min. The movement of the mice was video-tracked with Ethovision 11.5 software. Time of exploration of both objects (nose located in a 2 cm area around object) was automatically measured by the software. The traveled distance to reach the novel object or the same object (old object) was measured.

Three-chamber social preference test. The three-chamber apparatus consisted of a Plexiglas box (50x25 cm) with removable floor and partitions dividing the box into three chambers with 5-cm openings between chambers. The task was carried out in four trials. The three-chambers apparatus was cleaned and wiped with 70% ethanol between each trial and each three-chamber test experiments. The mouse was video-tracked using Ethovision software. In a 5 minutes habituation trial, the tested mouse was placed in the central part of the 3-chamber unit and freely explore the two empty wire cages located in the left and right chambers (duration in each chamber). In the second 10 minutes socialization trial, a “stranger” wild-type mouse (same sex and same age, S1) is placed randomly in one of the two wire cages. The second wire cage remains empty. Social recognition is analysed during the third 10 minutes trial, in which a second “stranger” mouse (S2) is placed in the second wire cage. The S1 mouse is still under the first wire cage and corresponds to the familiar mouse. Thus, the tested mouse will have the choice between a familiar mouse (S1) and a new stranger mouse (S2). After a 30 minutes delay, the M2 mouse is replaced by another “stranger” mouse

(S3) to test social memory. The measure of the real social contact is represented by the time spent in nose-to-nose interactions with the mouse. This test was performed using grouped-house mice.

Metabolic phenotyping

Animals were weighed at postnatal day 100 (P100) using an analytical balance. Perigonadal white adipose tissue was carefully dissected and weighed immediately after sacrifice using an analytical balance. Samples were fixed in 4% paraformaldehyde for 24 h at 4 °C and then dehydrated and paraffin-embedded using an automated tissue processor (Shandon Citadel™, Thermo Fisher Scientific). The dehydration sequence consisted of the following successive baths: 70% ethanol (1 h), 95% ethanol (1 h), 95% ethanol (1 h 30), absolute ethanol (1 h 30), absolute ethanol (2 h × 2), xylene (1 h), xylene (1 h 30), and paraffin (2 h 30 × 2). Paraffin used was Surgipath Paraplast (ref. 39601006), and xylene was Xylenes 98% pure (Acros Organics™, ref. 383930050).

Paraffin blocks were sectioned at 5 µm thickness using a Leica RM2245 microtome. Sections were collected on glass slides with a drop of water, stretched on a warm plate below the paraffin melting point (≤ 56 °C), and air-dried overnight at 35 °C. Slides were deparaffinized, rinsed in running water for 5 min, stained with Harris hematoxylin (Papanicolaou Harris hematoxylin, Bio-Optica, ref. 05-12011) for 3 min, rinsed again for 5 min, and counterstained with eosin Y 1% (1 g eosin Y, Acros Organics, ref. 152881000, in 100 mL Milli-Q water) for 3 min. Sections were dehydrated, cleared, and mounted with Eukitt. Slides were digitized using a Zeiss Axio Scan.Z1 automated slide scanner (Zeiss, Germany). Adipocyte morphology was quantified using ImageJ software (NIH, USA) with the home-made plugin to determine mean adipocyte area and size distribution.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism (GraphPad Software, Prism 7.0 software, Inc, La Jolla, CA, USA). All statistical tests were two-tailed and the level of

significance was set at $P<0.05$. Appropriate tests were conducted depending on the experiment; tests are indicated in the figure legends or detailed in supplementary statistical file. Values are indicated as Q2 (Q1, Q3), where Q2 is the median, Q1 is the first quartile and Q3 is the third quartile when non-parametric tests were performed and scatter dot plots report Q2 (Q1,Q3) or as mean ± SEM when parametric tests were performed usually in histograms. N refers to the number of animals, while n refers to the number of brain sections or hippocampi or cells recorded.

Mann-Whitney (MW) non-parametric test or t-test (parametric test) were performed to compare two matched or unmatched groups. ANOVA or Kruskal-Wallis tests were performed when the different groups have been experienced in the same experimental design only; if this was not the case, MW or t-test were used. One-way ANOVA followed by Bonferroni or Dunnett's or Tukey's post-hoc tests were used to compare three or more independent groups. Two-way ANOVA followed by Bonferroni post-hoc test was performed to compare the effect of two factors on unmatched groups. *: $p< 0.05$; **: $p <0.01$; ***: $p<0.001$; ****: $p<0.0001$. All the statistical analyses (corresponding to each figure) are reported in a specific file.

AUTHOR CONTRIBUTIONS

Conceptualization: FM, SB; Methodology and Investigation: FS, CS, MSA, PYB, FO, JLG, YB, FB, EP, SGB, FM; Validation: FS, CS, MSA, PYB, SGB, FM; Resource: FM, SGB; Writing: FM ; Visualization and Analysis: FS, PYB, MSA, CS, FO, JLG, YB, FM, SGB; Supervision: FM; Project administration: FM; Funding acquisition: FM and SGB. All the authors revised the manuscript.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

Table 1: Summary of the results

	Inhibition in neonates vs controls		**Inhibition in juveniles vs controls**		**Inhibition in young adults vs controls**	
Test	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
USVs	≠ Decreased		ND		ND	
Rotarod	=	=	=	=	=	=
Open Field (Time in Per vs Cen)	≠ (same time in both)	≠ (same time in both)	≠ (same time in both)	=	=	=
Open Field (distance)	=	=	=	=	≠ Decreased	=
Open field (grooming duration)	≠ Increased	=	=	=	=	=
Open field (rearing frequency)	=	=	=	=	≠ Decreased	=
NOR (time SO vs NO)	≠ (same time with both)	≠ (same time with both)	=	=	=	=
NOR (total exploration time)	=	=	≠ Decreased	=	=	=
Spontaneous Social Interaction	=	=	=	=	≠ Increased	=
3 chamber : Socialization	=	ND*	=	ND	=	ND
3 chamber: Social Novelty	≠ Decreased	ND	=	ND	=	ND
3 chamber: Social Memory	≠ Decreased	ND	≠ Decreased	ND	≠ Decreased	ND

- ND : Not Determined

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LEGENDS of FIGURES

Figure 1: Chemogenetic inactivation of OT-expressing neurons at three postnatal stages and performed studies.

a) Scheme representing the breedings and generations of cohorts producing OT-Cre::hM4Di-Ha (Cre+) and hM4Di-Ha (Cre-) mice injected with the DREADD agonist C21 at specific developmental stages. **b)** Immunohistochemistery performed on paraventricular nucleus (PVH) of hypothalamus of OT-Cre::Ai14::hM4Di-Ha transgenic mice showing the expression of OT (blue, ab anti-OT), the expression of the OT-cre allowing the recombination and expression of the tdTomato (red) and the expression of hM4Di-Ha (green, ab anti-Ha), in OT-expressing neurons only as shown by the overlap. Scale bar, 100 µm. **c)** Whole-cell current-clamp recordings of OT-expressing neurons expressing tdTomato and hM4Di in brain slices of pups (scheme), showing that application of C21 (10 µM, for 5 min) inhibits action potentials in Cre^+ pups but has no effect in Cre- pups. Spike frequency (Hz) of OT-expressing neurons recorded in the PVH in hM4Di/OT- Cre+ pups (Cre^+: N= 3, n=16; control: 2.95+- 0.3, C21: 0.71+- 0.2, Wash: 2.15 +-0.3) and Cre^- pups (Cre^+: N= 3, n=9; control: 3.93+- 0.3, C21: 3.98+- 0.5, Wash: 3.78 +-0.3). RM one-way ANOVA followed by Tukey's multiple comparison. **d)** The studied mouse cohorts with inhibition at specific periods and tests performed for the different cohorts. (***P<0.001, ****P<0.0001).

Figure 2 : **Behaviour of mice following chemogenetic inhibition of OT-expressing neurons in infancy (P0-P7).**

a) All cohorts of mice with subcutaneous injection of C21 in infancy (P0-P7). **b)** Number of ultrasonic vocalisations (USVs) of Cre+ and Cre- pups (all males and females) at P8 when they are separated from their mothers. **c)** Results of the rotarod test (latency to fall) performed in males and females Cre+ and Cre- during three following days. **d-g)** Results of the open field test performed in males and females Cre+ and Cre-: time spent in periphery (Per) versus central (Cen) zone (**d**); travelled distance (**e**); grooming duration (**f**) and rearing frequency (**g**).

h-i) Results of the novel object recognition (NOR) test in males and females Cre+ and Cre-: time of interaction with the familiar (same object, SO) or novel object (NO) (**h**) and total time of exploration of both objects (**i**). **j)** Spontaneous social interaction in an OF: time spent to interact between a tested mouse with a juvenile one (same sex) in males and females Cre+ and Cre-. **k)** Results of the 3 Chamber test in Cre+ and Cre- males : time spent in each chamber for the habituation or sniffing times spent interacting with each restrained mouse in the socialization, social novelty or social memory tests are measured.

Data are presented as mean ± SEM (rotarod) or median + quartiles (all other tests). Statistical significance between groups was determined by a Mann-Whitney test (b, e, f, g, i, j), ANOVA with Bonferroni multiple comparison test (c), Wilcoxon test (d, h) or a repeated measures ANOVA (Mixed effects analysis) with Sidak's multiple comparison test (k). (NS: not significant, *P <0.05, ** P <0.01, ***P<0.001).

Figure 3: Behaviour of mice following chemogenetic inhibition of OT-expressing neurons during male or female puberty (juveniles).

a) All cohorts of mice treated with C21 during female (P22-P28) or male puberty (P28-P34). **b)** Results of the rotarod test (latency to fall) performed in males and females Cre+ and Cre-. **c-f)** Results of the open field test performed in males and females Cre+ and Cre-: time spent in periphery versus central zone (**c**) ; travelled distance (**d**) ; grooming duration (**e**) and rearing frequency (**f**). **g-h)** Results of the novel object recognition (NOR) test in males and females Cre+ and Cre-: time of interaction with the familiar (same object, SO) or novel object (NO) (**g**) ; total time of exploration of both objects (**h**). **i)** Spontaneous social interaction in an OF : time spent to interact between a test mouse with a juvenile one (same sex) in males and females Cre+ and Cre-. **j)** Results of the 3 Chamber test in males and Cre+ and Cre-: time spent in each chamber for the habituation or sniffing times spent interacting with each restrained mouse in the socialization, social novelty or social memory tests are measured.

Data are presented as mean ± SEM or median + quartiles. Statistical significance between groups was determined by a Mann-Whitney test (b, d, e, f, h, i), ANOVA with Bonferroni multiple comparison test (b), Wilcoxon test (c, g) or a repeated measures ANOVA (Mixed effects analysis) with Sidak's multiple comparison test (j). (NS: not significant, *P <0.05, ** P <0.01, ***P<0.001).

Figure 4: Behaviour of mice following chemogenetic inhibition of OT-expressing neurons in young adults (P56-P62).

a) All cohorts of mice treated with C21 during adulthood (P56-P62) **b)**. Histograms of the results of the rotarod test (latency to fall) performed in males and females Cre+ and Cre-. **c-f)** Results of the open field test performed in males and females Cre+ and Cre-: time spent in periphery versus central zone (**c**) ; travelled distance (**d**) ; grooming duration (**e**) and rearing frequency (**f**). **g-h)** Results of the novel object recognition (NOR) test in males and females Cre+ and Cre-: time of interaction with the familiar (same object, SO) or novel object (NO) (**g**) ; total time of exploration of both objects (**h**). **i)** Spontaneous social interaction in an OF : time spent to interact between a test mouse with a juvenile one (same sex) in males and females Cre+ and Cre-. **j)** Results of the 3 Chamber test in males and Cre+ and Cre-: time spent in each chamber for the habituation or sniffing times spent interacting with each restrained mouse in the socialization, social novelty or social memory tests are measured.

Data are presented as mean ± SEM or median + quartiles. Statistical significance between groups was determined by a Mann-Whitney test (b, d, e, f, h, i), ANOVA with Bonferroni multiple comparison test (b), Wilcoxon test (c, g) or a repeated measures ANOVA (Mixed effects analysis) with Sidak's multiple comparison test (j). (NS: not significant, *P <0.05, ** P <0.01, ***P<0.001).

Figure 5 : Consequences of chemogenetic inhibition of OT-expressing neurons during infancy, juvenile and in young adults on body weight and body composition.

(a,d) Body weight ; **(b,e)** Weight of the perigonadal adipose tissue (fat mass) and **(c,f)** average size of perigonadal adipocytes in 100-days-old Cre+ and Cre- males **(a,b,c)** and females **(d,e,f)** treated with C21 during neonatal or juvenile or adult periods. All data normalized to Cre-. **(g)** Representative images of Hematoxylin Eosin staining of the perigonadal adipose tissue, scale bar 100µm. **(h)** Adipocyte size distribution in 100-days-old and Cre+ or Cre- male and female mice treated with C21 neonatally or during the juvenile period. Data are expressed as means ± SEM. Statistical analysis was performed using t-test (*P <0.05, ** P <0.01, ***P<0.001).

Figure 6 : Impact on neonates following chemogenetic inhibition of OT-expressing neurons in foetuses from gestational day 18 to the day of birth.

a) Treatment of dams with C21 in the bottle from day 18 of gestation (GD18) until birth. **b)** Number of cFos-positive cells in the whole PVH of Cre+ and Cre- neonates (2 h after birth) issued from the same litter, to assess inhibition of OT-expressing neurons in neonates. **c)** Delivery day of pregnant females treated with C21 and with litters containing only Cre- neonates or a mixture of Cre+ and Cre- neonates. **d)** Delivery day of pregnant females treated with C21 and with litters containing a mixture of Cre+ and Cre- neonates with over 50% of Cre+ or less than 50% of Cre+. **e)** Survival and feeding status (milk presence in stomach) of Cre+ and Cre- neonates (from C21-treated mothers) 12-24h after birth: presence of milk in the stomach estimated by eye. Statistical analysis was performed using Chi^2-test (*P <0.05, ** P <0.01, ***P<0.001).

Figure 1: Chemogenetic inactivation of OT-expressing neurons at three postnatal stages and performed studies.

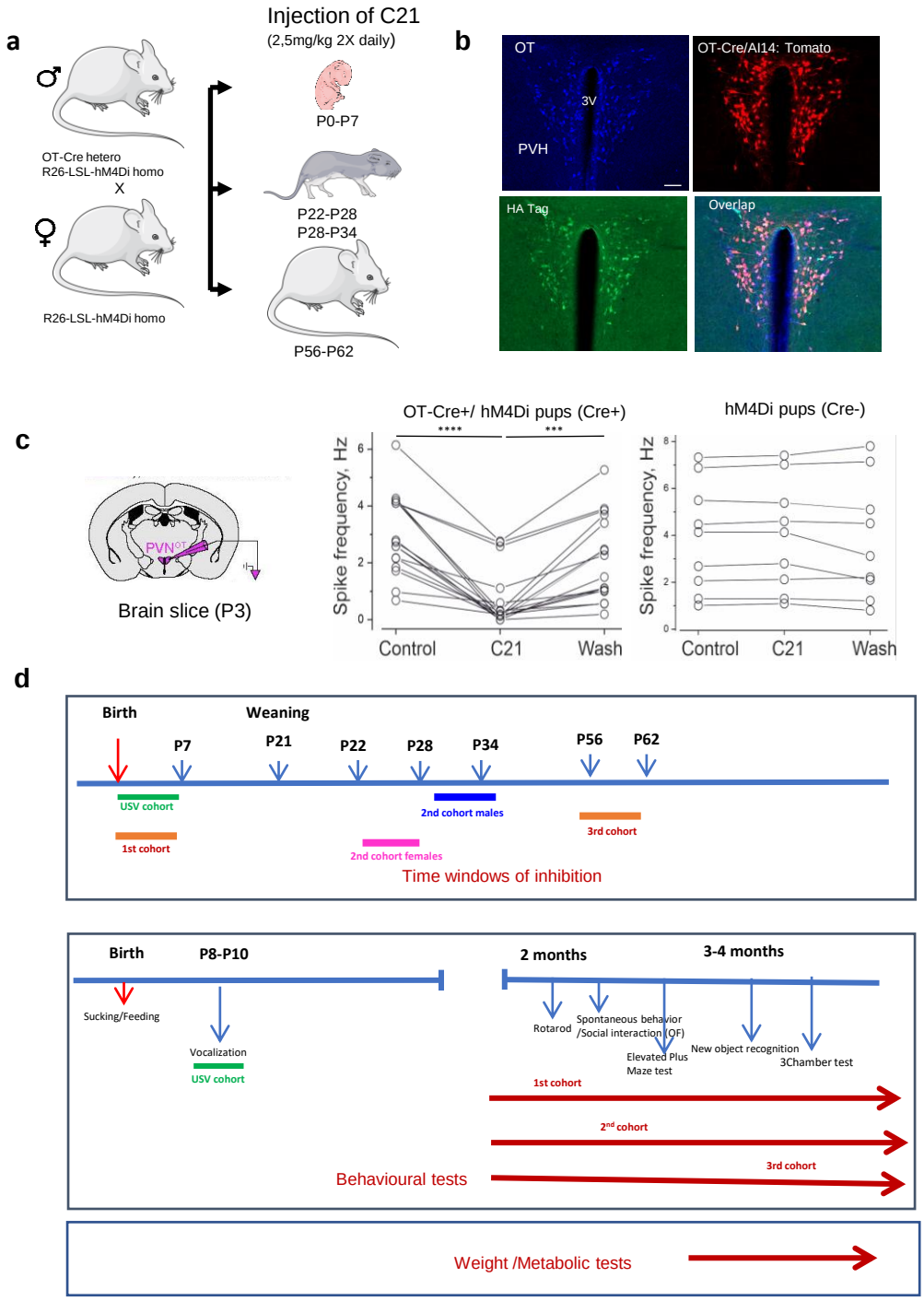


Figure 2: Behaviour of mice following chemogenetic inhibition of OT-expressing neurons in infancy (P0-P7).

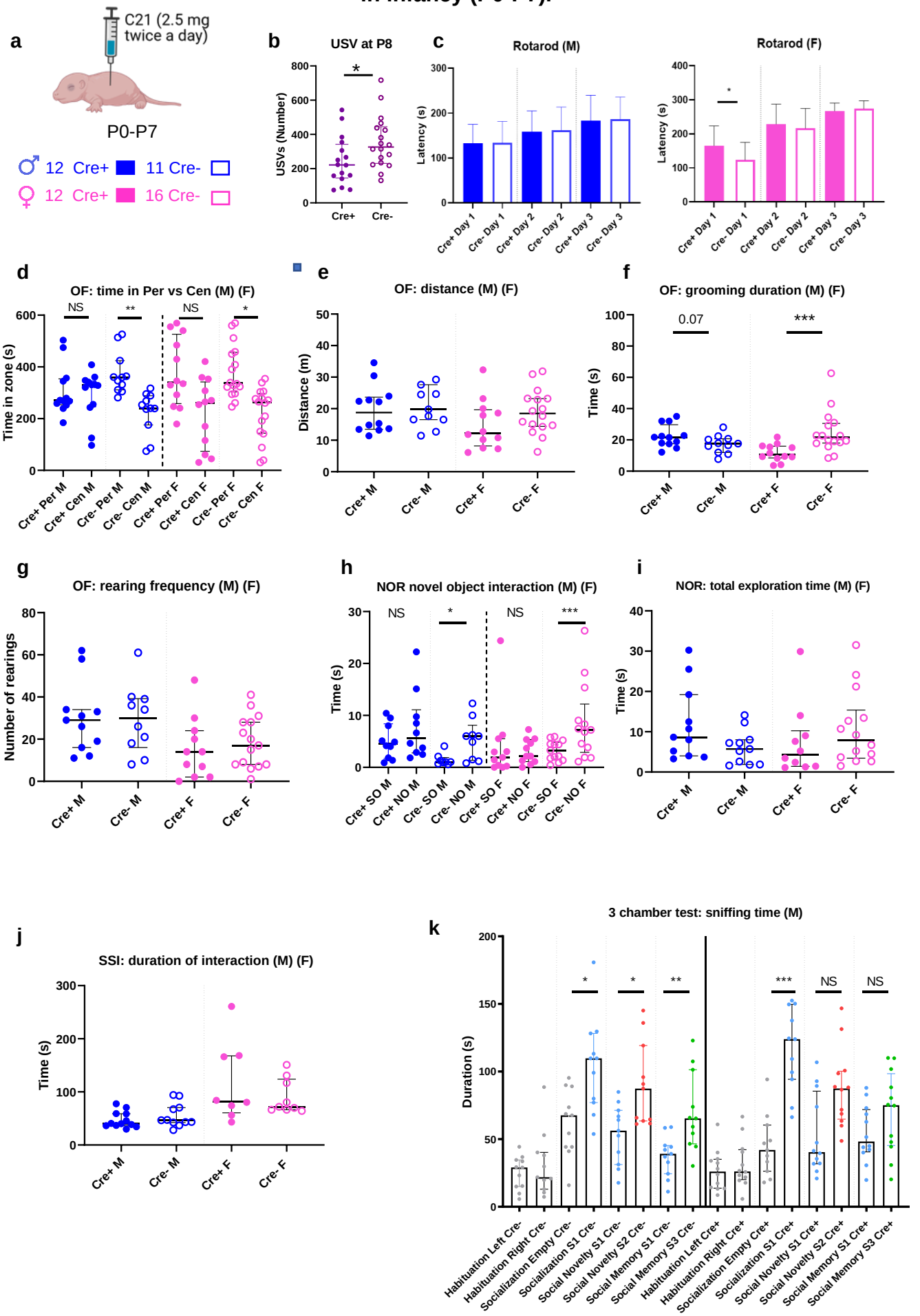


Figure 3: Behaviour of mice following chemogenetic inhibition of OT-expressing neurons during male or female puberty (juveniles).

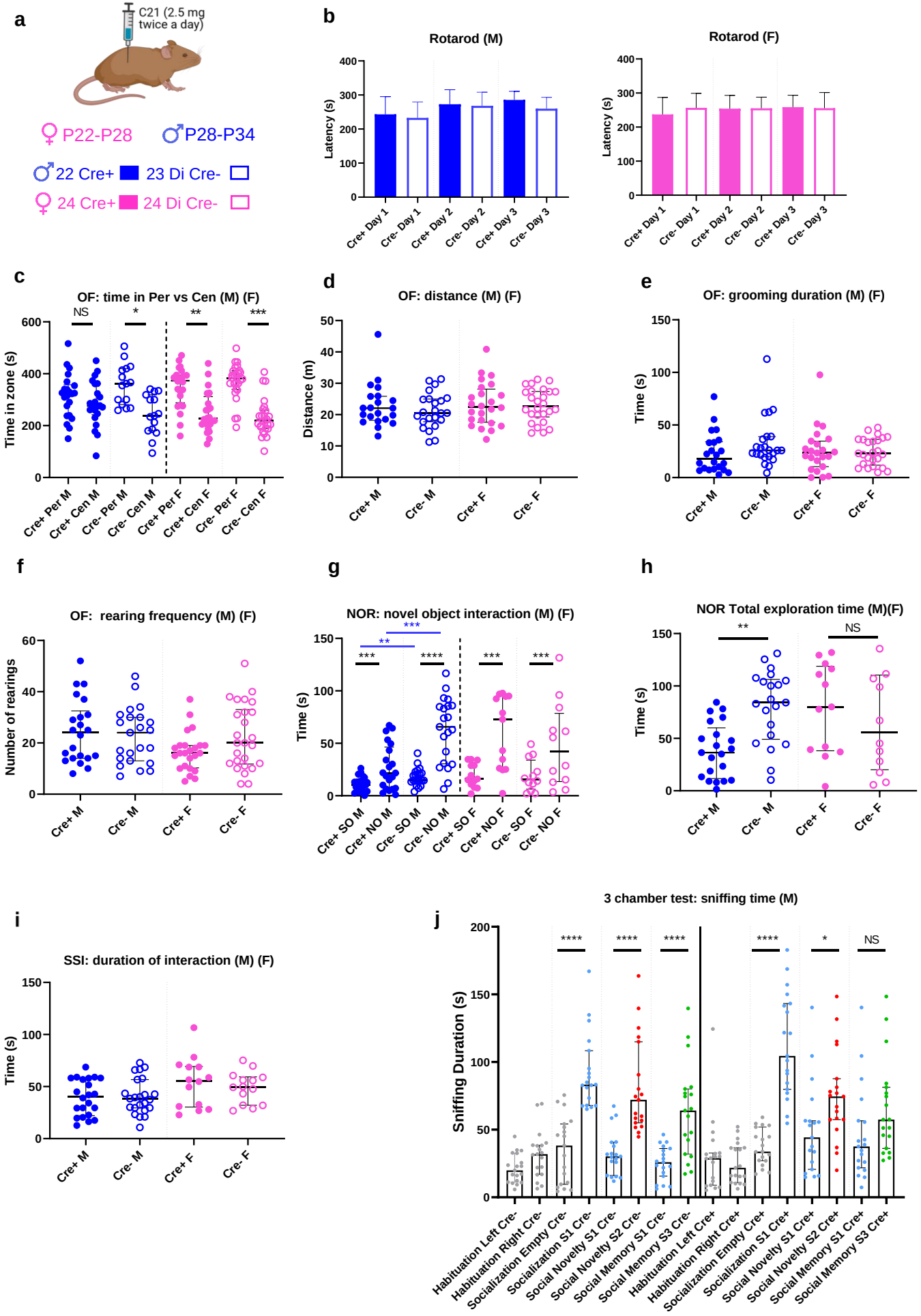


Figure 4: **Behaviour of mice following chemogenetic inhibition of OT-expressing neurons in young adults (P56-P62).**

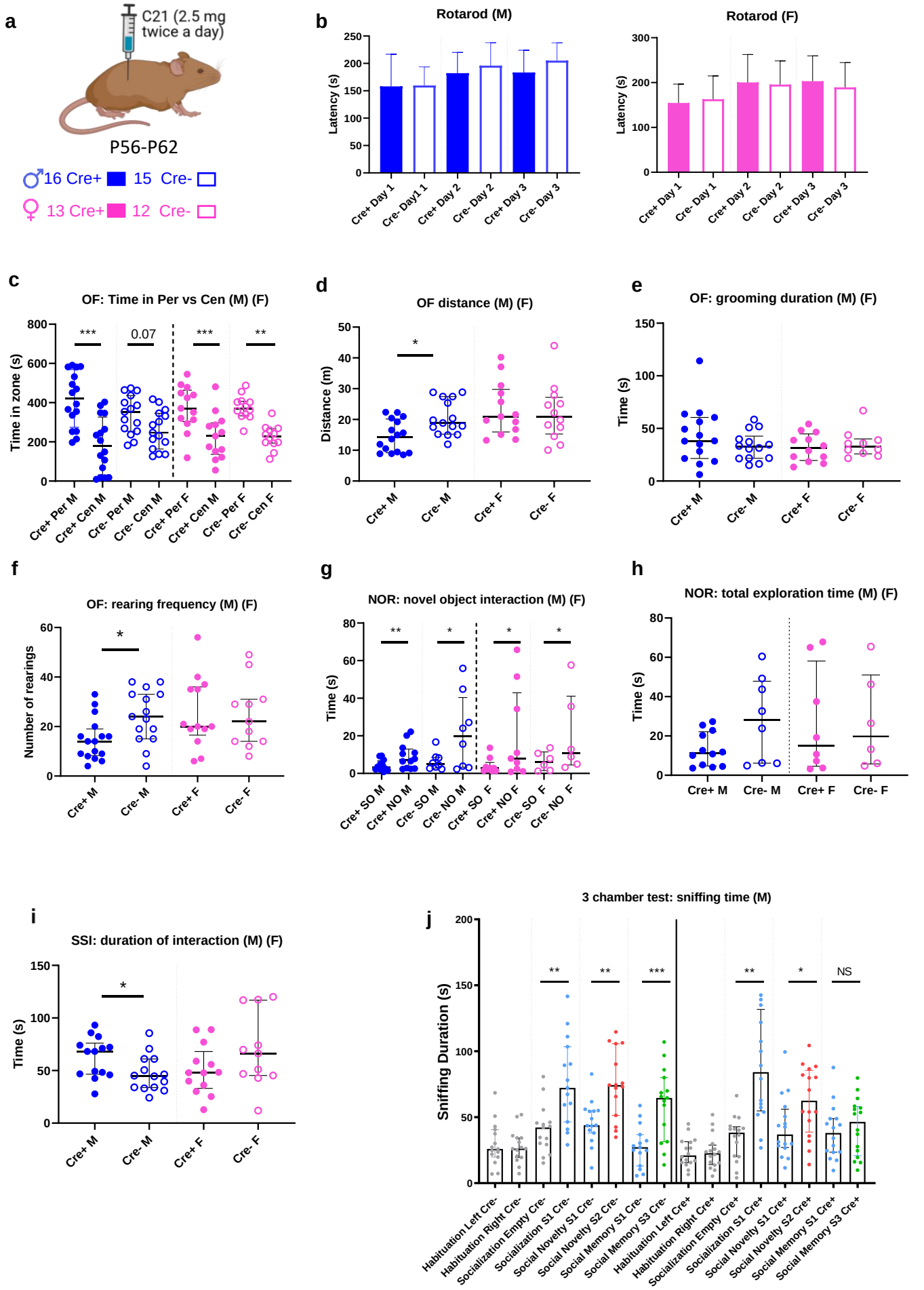


Figure 5: **:Consequences of chemogenetic inhibition of OT-expressing neurons during infancy, juvenile and in young adults on body weight and body composition.**

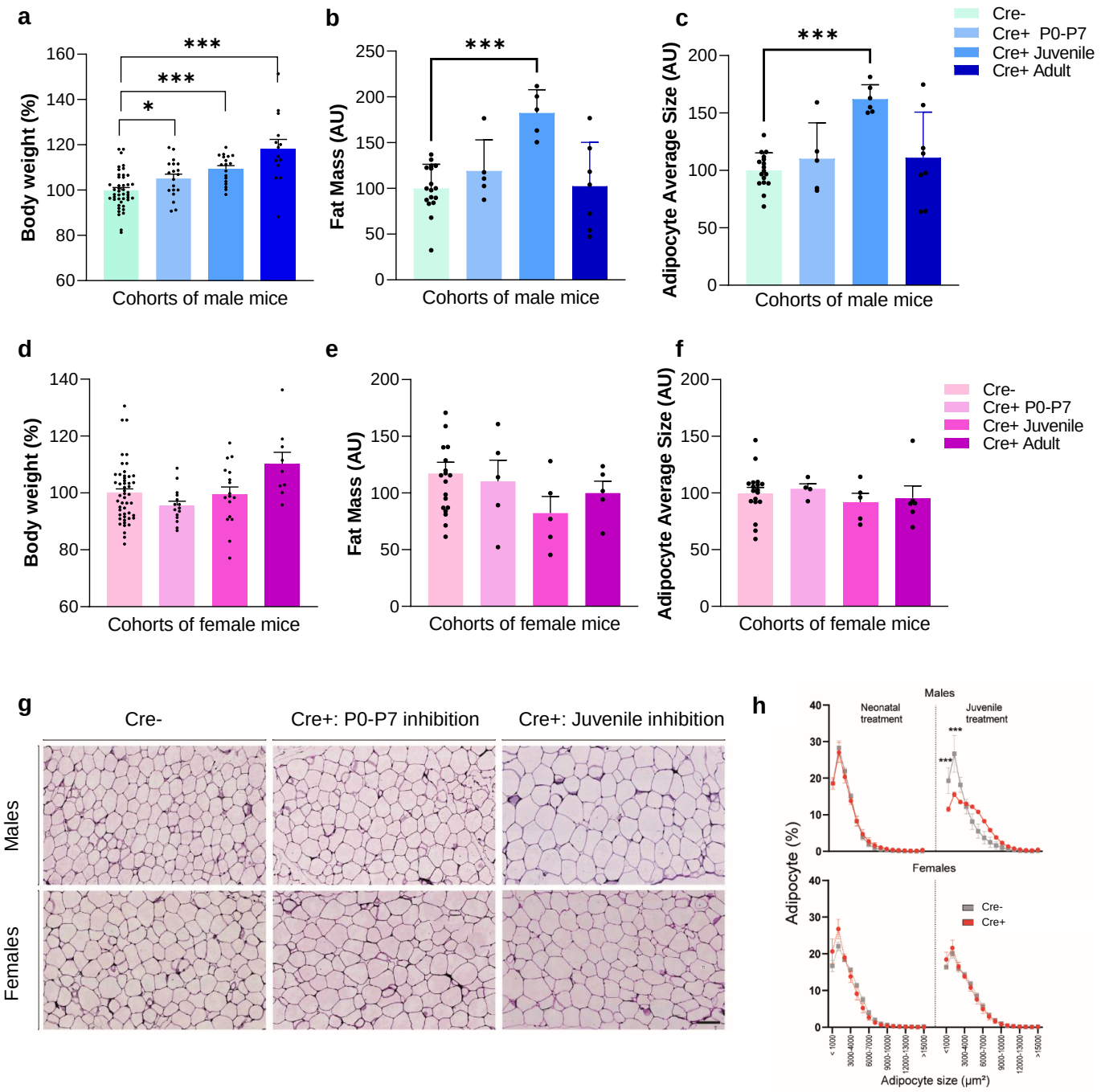
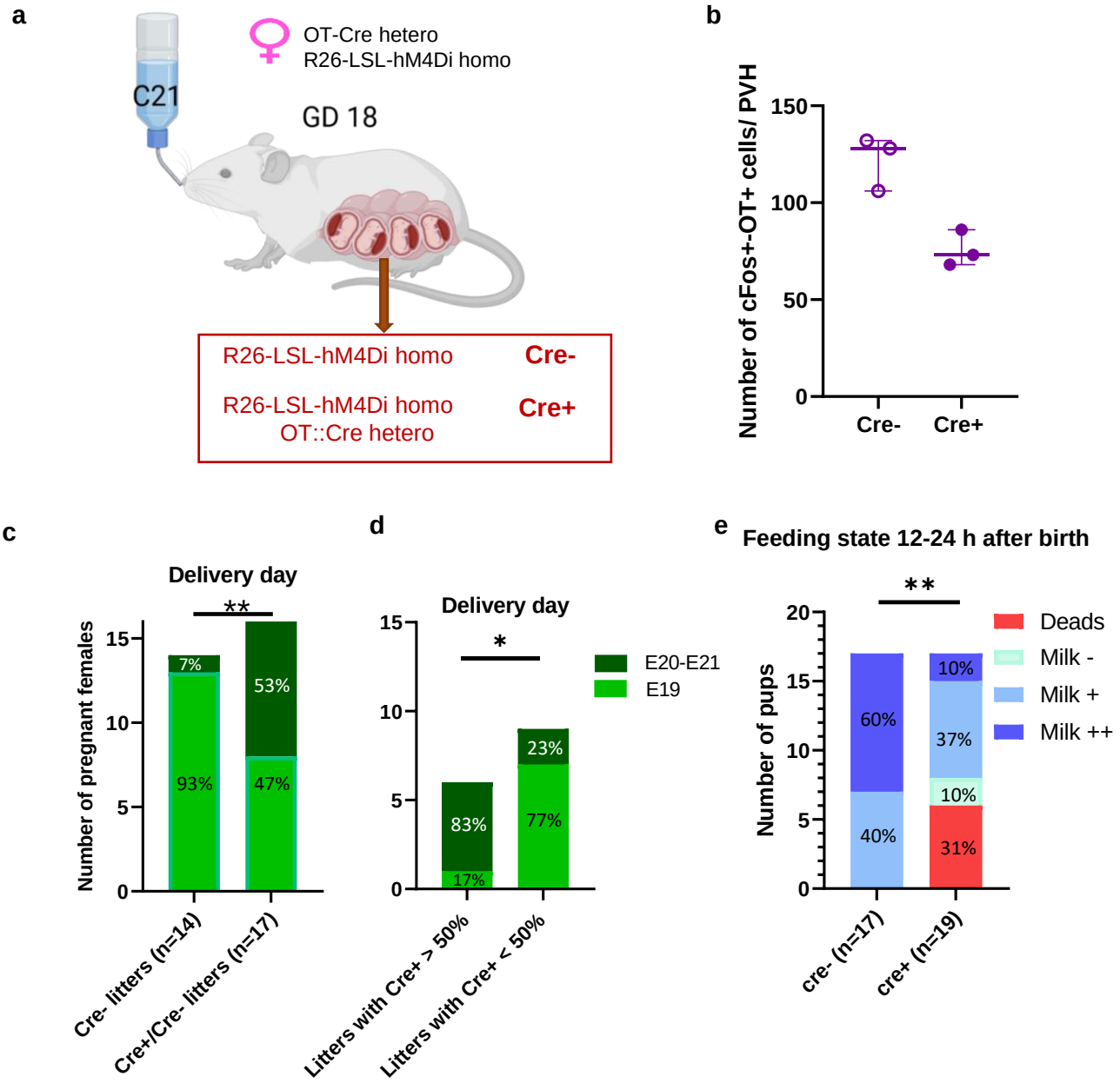


Figure 6: Impact on neonates following chemogenetic inhibition of OT-expressing neurons in foetuses from gestational day 18 to the day of birth.



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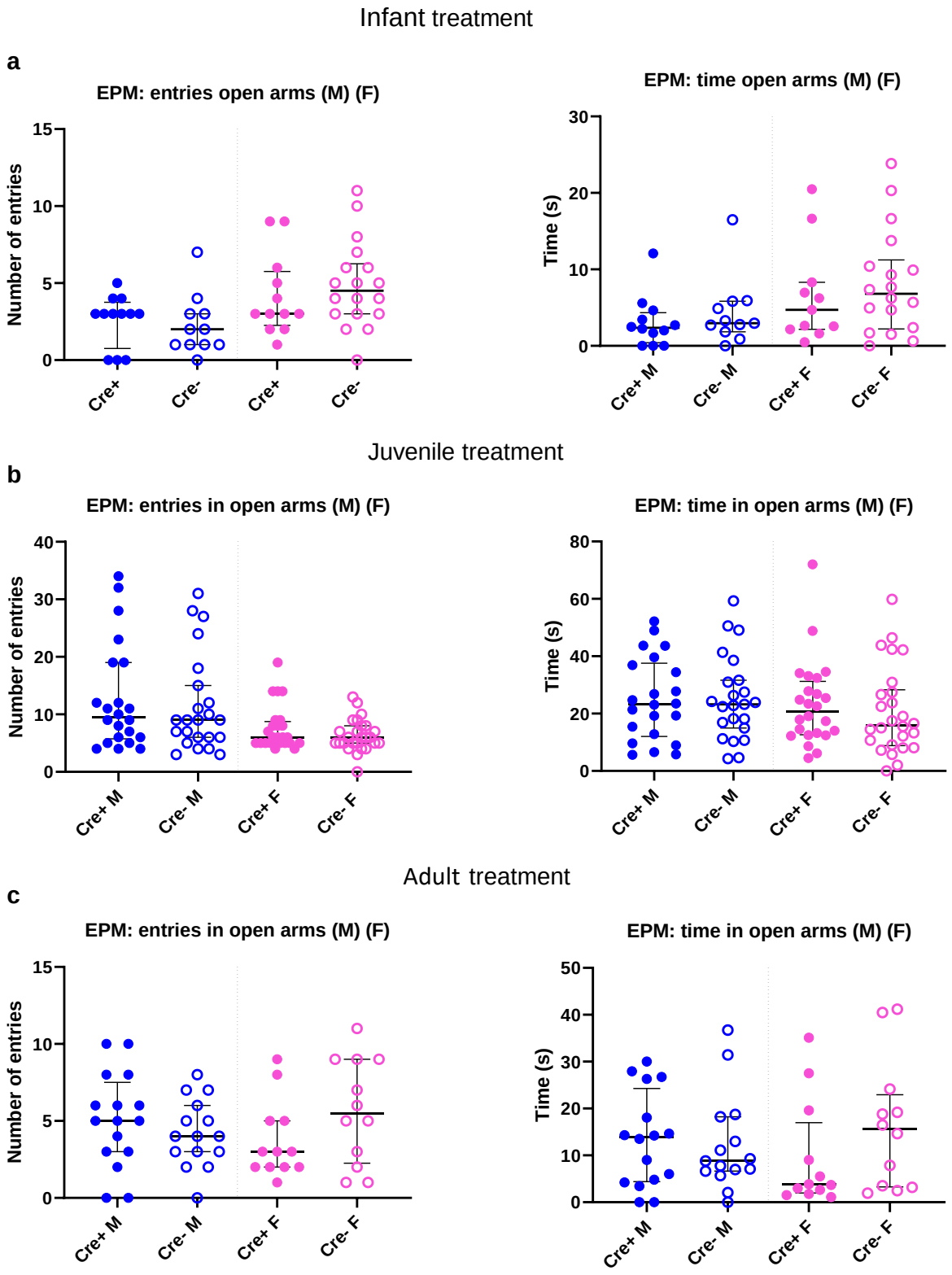
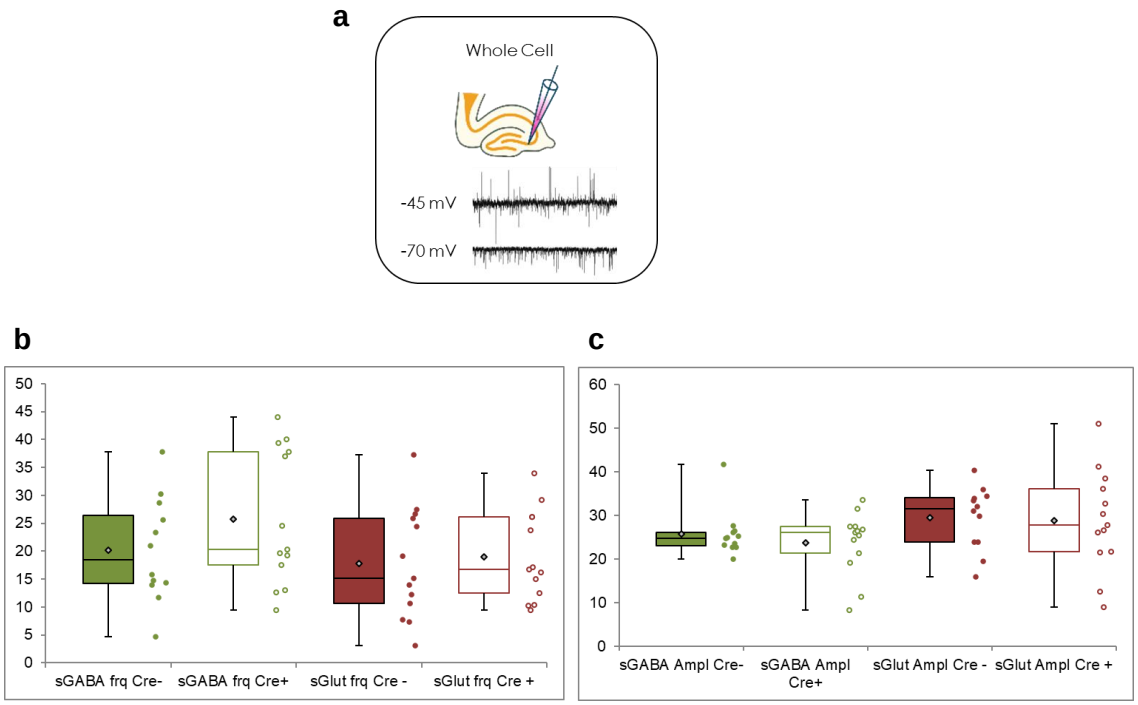
Supplementary Figures



Supplementary Figure 1: Elevated Plus Maze tests on the different cohorts of male and female mice following chemogenetic inhibition of OT-expressing neurons. Number of entries in open arms and time spent in open arms are assessed following inhibition in neonates (a), in juveniles (b) and in young adults (c). Data are presented median + quartiles. Statistical significance between groups was determined by a Wilcoxon Mann-Whitney test.



Supplementary Figure 2:**Supplementary Figure 2**: **Spontaneous Glutamatergic and GABAergic synaptic activity of CA3 pyramidal neurons in the anterior hippocampus region in mice following chemoinhibition of OT-expressing neurons during infancy (Cre+) compared to controls (Cre-) mice.**

a) Paradigm of whole cell recordings performed at a holding potential of -45 mV (sGABA) or -70 mV (sGlut). The glutamatergic synaptic currents are inwards and the GABAergic synaptic currents are outwards. **b-c)** Values in the different cohorts of the Glut-sPSCs(b) and GABA-sPSCs (c) frequency and amplitude and amplitude. *Cre*+ (N= 4, n=13), Cre- (N=4, n=12), have been analyzed, with N: number of mice and n: number of recorded cells. Data represented in whisker-plots report the different values of recorded cells with mean ±SEM, with scattered plots showing individual data points: sGABA frq Cre- (20.1 ± 2.7); sGABA frq Cre+ (25.7± 3.3); sGlut frq Cre- (17.8 ± 2.8); sGlut frq Cre+ (18.9 ± 2.2); sGABA Ampl Cre- (25.7± 1.5); sGABA Ampl Cre+ (23.8 ± 2); sGlut Ampl Cre- (29.5± 2); sGlut Ampl Cre+ (28.8 ± 3.1).

Statistical significance between groups was determined one-way ANOVA + Tuckey *post hoc* test.

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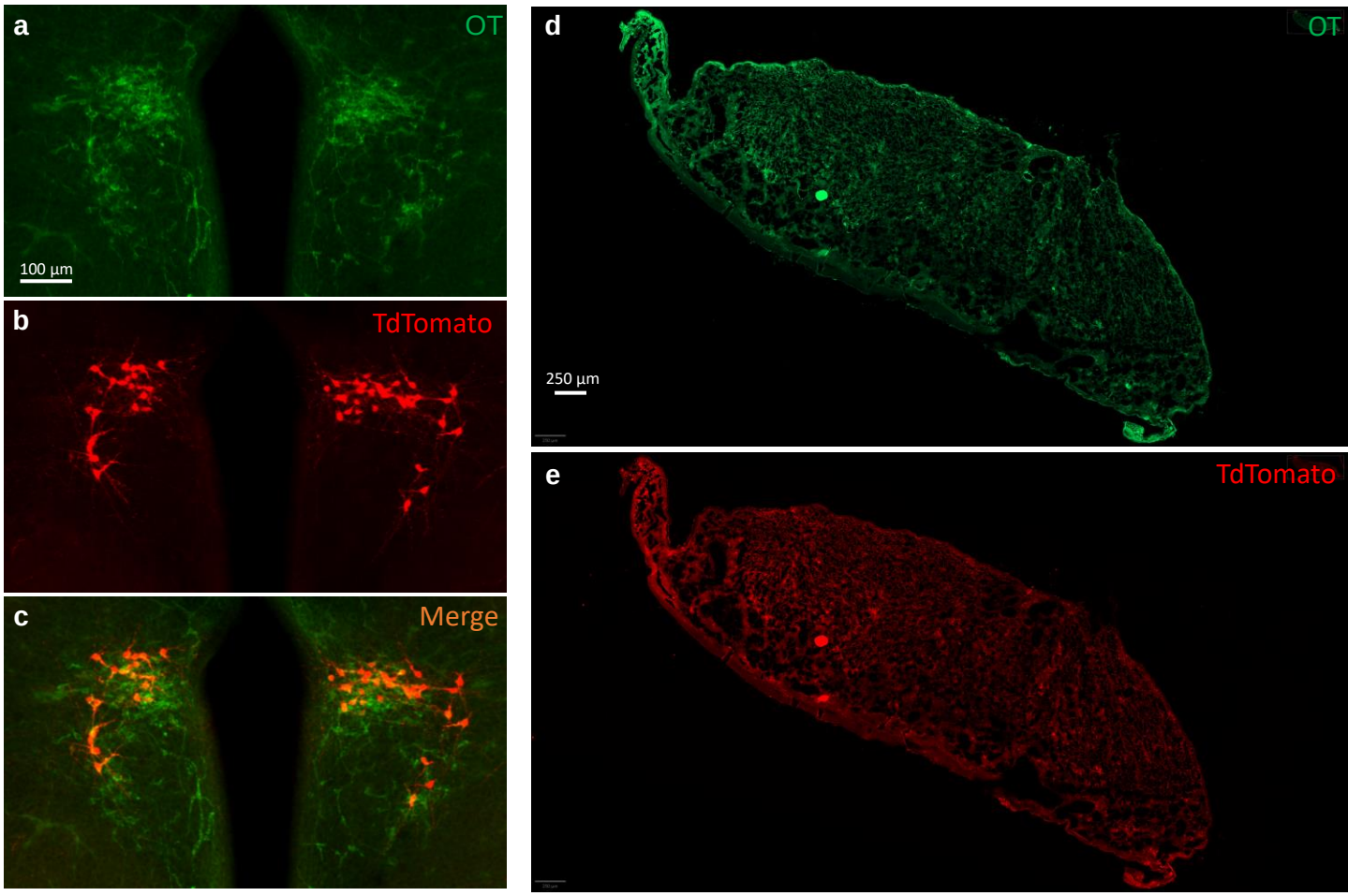

Supplementary Figure 3: **Expression of fœtal oxytocin in brain and placenta of E18.5 OT-Cre::Ai14 foetuses.** We used OT-Cre::Ai14 foetuses generated by crossing one Ai14 (RCL-TdTomato) female with one OT-Cre male in order to report OT expression via the fluorescent Tomato in fœtal placenta or brain but not in the mother tissues. OT expression, whatever the mother or fœtal origin, is detected by an OT-antibody -green). a,d : immunohistochemistery using an OT-antibody on brain (a) and placenta of E18.5 foetuses (d). b-e : immunohistochemistery using a Tomato-antibody on brain (b) and placenta (e) of E18.5 foetuses. c : merge in the brain.