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Early life oxytocin treatment improves thermo-sensory reactivity and maternal behavior in neonates lacking the autism-associated gene Magel2

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INTRODUCTION

Autism spectrum disorder (ASD) is a developmental disorder characterized by challenges with social interaction, speech and non-verbal communication, as well as repetitive behaviors. However, atypical sensory behaviors are a core aspect of ASD affecting 90% of children [1]. Importantly, atypical sensory sensibilities have been documented as early as 6 months in ASD infants, preceding considerably the common core features and the diagnosis. Recent increasing evidences suggest that sensory traits such as tactile, visual, auditory, olfactory, gustatory and heat abnormalities are present in juvenile and adult ASD models [2–9].

During the first week of life, sensory integrity is instrumental since neonates have to undertake vital innate behaviors such as nipple-searching and alert calls. Among the various stimuli arising from the external world, sensing any reduction of the ambient temperature is particularly relevant for neonatal pups. Indeed, unlike their homeothermic adult counterparts, neonates are poikiloithermic [10] and should be kept in close contact with the mother or their littermates in order to keep their body temperature. In the absence of their warmth-giving mother and being exposed to cool temperatures, neonate mice generate ultrasonic vocalizations (USV). In fact, during the perinatal period, exposure to low ambient temperatures is considered as a major stimulus eliciting USV [11–13]. Interestingly, atypical early vocalization has been detected in 6-month-old infants at risk for autism [14] and may represent an early biomarker for ASD [15].

The neuronal pathways underlying cool response behavior are still under intensive investigation. At the peripheral level, thermosensory neurons have been described in the skin and in the Grueneberg ganglion (GG) – a cluster of sensory neurons localized at the tip of the nose [16–19]. It has been proposed that this ganglion influences USV [17, 20] generated by rodent neonates to elicit maternal care on exposure to cool temperatures [11, 13, 21].

Following cool exposure, newborns require an effective thermoregulatory adaptive response to produce heat [22]. During this period of development, they are unable to shiver and the primary source of heat is produced by the sympathetically mediated metabolism of brown adipose tissue (BAT); named non-shivering thermogenesis [23].

Here, using behavioral tests to assess neonatal thermosensory reactivity, we discovered the existence of early developmental deficits in thermal sensitivity in neonate mice lacking the autism-associated gene Magel2. MAGEL2 is an imprinted gene highly expressed in the hypothalamus that is paternally expressed and Schaaf-Yang [25] respectively; two syndromes with high prevalence of ASD (27% and 78% respectively). The patients have sensory disorders characterized by instability of body temperature.
manifested with episodes of hypothermia without infectious causes and which can be fatal in infants [26, 27]. Moreover, adolescent with ASD present a decreased sensitivity to thermal stimuli [28].

With the aim to explore the physio-pathophysiologic mechanism underlying this thermal deficit we explored peripheral functional activities of both GG and BAT. Furthermore, since the oxytocinergic system is considered as a rheostat of adult sensory functions [29], a modulator of huddling and thermotaxis behavior in response to cold challenge [30] and it is altered in Magel2+/−/− pups [31, 32], we investigated whether central dysfunction of the oxytocinergic system could sustain neonatal thermosensory reactivities and whether neonatal oxytocin (OT) pharmacological treatment could be a therapeutic approach.

Finally, whilst OT is indeed important for maternal care behavior [33], in this study the manipulation of temperature and OT occurs only in the pup, there were no modification of OT in the dam which are all WT.

MATERIAL AND METHODS

Animals

Mice were handled and cared 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 22nd, 2010 (2010/63/EU). Experimental protocols were approved by the institutional Ethical Committee Guidelines for animal research with the accreditation no. B13–055–19 from the French Ministry of Agriculture. All efforts were made to minimize the number of animals used. 129-Gt(ROSA)26Sor-J/Ftm-1Cre/+ (C57BL/6J, hereafter referred to as hM4Di DREADD homozygous // heterozygous OT−/− mice were obtained from the Jackson Laboratory (stock #24234). In our experiment we used hM4Di DREADD homozygous // heterozygous OT−/− mice for convenience. Due to the parental imprinting of Magel2 only heterozygous pups (+/m+/−) with the mutated allele transferred from the male were used for experiments. The OT−/− mice were obtained from the Jackson Laboratory (stock #24234).

In our experiment we used hM4Di DREADD homozygous // heterozygous OT−/− mice (here referred as OT hM4Di).

Neonatal thermoregulatory behavior and USV recording

On the day of testing (P0, P1, P2, P3 and P6), after 30 min of room habituation in the testing room, each pup was separated from its littermates and dam, placed on a heating pad and each pup was isolated in a box (23 × 28 × 18 cm) located inside an anechoic box (54 × 57 × 41 cm; Couldbourn instruments, PA, USA) for a 5 min test at room temperature (25 °C). Then the pup goes back to the dam for 5–10 min and is submitted to a second separation, placed on a heating pad and the USV were recorded following 5 min under cool temperature (17 °C). We conducted a reversal assay (17 °C then 25 °C) to exclude any potentiation effect. An ultrasound microphone (Avisoft UltraSoundGate condenser microphone CM16/CMPA, Avisoft bioacoustics, Germany) sensitive to frequencies of 10–250 kHz in 16 bits format. Data were transferred to SASLab Pro software (version 5.2; Avisoft bioacoustics) and a fast Fourier transform was performed. Data were analyzed by Bio-Rad, and densitometric analyses were performed using the ImageLab™ software version 5.0 (Bio-Rad) to determine relative Triglyceride content per sample.

2-photon calcium imaging

GG slices of 400 µm were prepared from fresh tissue. Animals were euthanized by decapitation; the nose was dissected and bathed into a vibratome chamber (Leica) containing oxygenated (95% O2 and 5% CO2) aCSF. aCSF composition was as follows (in mM): 126 NaCl, 3.5 KCl, 2 CaCl2, 1.3 MgCl2, 1.2 Na2HPO4. 25 NaHCO3 and 11 glucose, pH 7.4 equilibrated with 95% O2 and 5% CO2. GG slices were incubated with 10 µM of Fura-2-AM (Life technologies) added with Pluronic acid and dissolved in DMSO, and 10 min and is submitted to a second separation, placed on a heating pad and the USV were recorded following 5 min under cool temperature (17 °C). We conducted a reversal assay (17 °C then 25 °C) to exclude any potentiation effect. An ultrasound microphone (Avisoft UltraSoundGate condenser microphone CM16/CMPA, Avisoft bioacoustics, Germany) sensitive to frequencies of 10–250 kHz in 16 bits format. Data were transferred to SASLab Pro software (version 5.2; Avisoft bioacoustics) and a fast Fourier transform was performed. Data were analyzed by Bio-Rad, and densitometric analyses were performed using the ImageLab™ software version 5.0 (Bio-Rad) to determine relative Triglyceride content per sample.
warm with aCSF for 1 min. Images were acquired every 5 s with an Olympus BX61WI microscope equipped with a multibeam multiphoton pulsed laser scanning system (LaVision BioTecs) as previously described [38]. Images were acquired through a CCD camera, which typically resulted in a time resolution of 50–150 ms per frame. Slices were imaged using a 20×, NA 0.95 objective (Olympus). Images were collected by CCD-based imaging system running ImspectorPro software (LaVision Biotec) and analyzed with Fiji software [39].

**Protein extraction and western blotting**

Brain and brown adipose tissues were homogenized in RIPA buffer (Thermo Fisher Scientific) with phosphatase and protease inhibitor cocktails (Pierce Protease and Phosphatase Inhibitor Mini Tablets, EDTA-Free) added with 1% Triton (Euromedex, life sciences products) for the brown adipose tissues. Proteins were run on a polyacrylamide gel (Bolt 4–12% Bis Tris plus, Invitrogen by Thermo Fisher Scientific) and transferred to a nitrocellulose membrane (GE Healthcare Life Science). Primary
antibodies were incubated overnight at 4 °C and were as follows: UCP1 (1:1000, Cell Signalling technology, #14670); p44/p42 MAPK (1:1000, Cell Signalling technology, #9102); phospho-p44/p42 MAPK (1:1000, Cell Signalling technology, #9101); GAPDH (1:1000, Invitrogen#PA9187). Signals were detected using Super Signal West Pico (Thermo Fisher Scientific, #34080) and bands were analyzed with ImageJ.

Reverse transcription and real time quantitative PCR

Wild-type and mutant newborns were sacrificed at P2 (between 2 pm and 4 pm). The hypothalamus, BAT and dorsal root ganglia (DRG) tissues were quickly dissected on ice and rapidly frozen in liquid nitrogen, then stored at −80 °C. Total RNA was isolated using the RNeasy® Mini Kit (Qiagen, cat #74104), according to the manufacturer’s protocol and cDNAs were obtained by reverse transcription using QuantiTect® Reverse Transcription Kit (Qiagen, cat #205311), starting with 600 ng of total RNA.

Statistical analysis

Statistical analyses were performed using GraphPad Prism (GraphPad Software, Prism 8.0 software, Inc, La Jolla, CA, USA). All the statistical analyses are reported in legends. Values are indicated as follows: (Q2 (Q1, Q3) or mean ± SEM, p-value, statistical test) where Q2 is the median, Q1 is the first quartile and Q3 is the third quartile.

RESULTS

ASD-related Magel2 mutation leads to neonatal thermosensory behavior alterations and impairment of maternal pup retrieval during the first week of life

To assess thermosensitivity in neonates, we developed an experimental procedure based on coolness-induced USV [17] (Fig. 1A). Wild-type (WT) and Magel2−/− (i.e., Magel2 KO) neonates aged from 0 to 6 days old (P0 to P6) were taken from their nests, isolated from the dam, and exposed separately and successively to two different temperatures (ambient: 25 °C and cool: 17 °C). Analyzing the latency in emitting the first call, which reflects the reactivity of the animals to sense cold, we found that WT neonates presented a lower latency at cool temperatures than under ambient exposure, while Magel2−/− did not (Fig. 1B and Supplemental Fig. 1A, C, E).

Furthermore, the proportion of neonates responsive to cooling was markedly decreased in Magel2−/− from P1 to P6 compared to the WT (Fig. 1C and Supplemental Fig. 1B, D, F). Comparison of the latencies (Fig. 1D, E) between WT and Magel2−/− revealed a significant age-dependent difference under cool (Fig. 1E) but not ambient exposure (Fig. 1D). We found that this atypical sensory reactivity was not the result of a motor/vocalization deficit since the number of USV and latency of the first calls were not affected under ambient temperature condition (Fig. 1D and Supplemental Fig. 1G). However, the number of calls were significantly reduced under cool exposure (Supplemental figure H). This deficit was independent of the sex (Supplemental Fig. 1G, H). We also found that dam separation and handling did not affect corticosterone levels of Magel2−/− and WT in both male and female and cannot be linked to this atypical thermosensory reactivity (Supplemental Fig. 2A, B).

To exclude any potentiation of dam separation, we ran assays in which USV box temperature was kept at 25 °C during the two periods of USV recording (Supplemental Fig. 3A). Using new cohorts of animals, we found that WT performed comparably upon repetitive ambient temperature exposures (Supplemental Fig. 3B, C, G, H). Moreover, we ran experimental paradigm inversion in which another cohort of animals was first exposed to 17 °C and then to 25 °C (Supplemental Fig. 3D). WT neonates still presented a low-latency response when exposed first to cool versus ambient temperatures (Supplemental Fig. 3E, D, F, G, H).

Thus, the latency of the first call of isolated neonates was dependent on external temperature. As previously observed, we confirmed that USVs responses to a cool challenge decreased as the number of unretrieved pups increased. WT pups retrieved between 25 °C and 17 °C environmental conditions: WT 25 °C: 14.86 ± 7.41; p 78.69 ± 18.59 s. Before/after graphs illustrating the latency to the first call of isolated pups by a wall in a way that the dam can only ear and respond to the ultrasonic vocalizations (USV) emitted by the pups (Fig. 1B and Supplemental Fig. 1G).
pups, we measured every 30 s throughout the test the proportion of retrieved pups. We observed that less than 15% of WT pups were retrieved during the first 30 seconds of the test in ambient condition while under cool condition this proportion was significantly increased to 50% (Fig. 1H). In contrast, at the same time, the proportion of retrieved $\text{Magel2}^{+/p}$ pups under cool temperature was only 10% (Fig. 1I). Furthermore, more than a third of $\text{Magel2}^{+/p}$ pups were not retrieved which was not observed for WT pups in cool condition (Fig. 1I). We found no difference between male and female of those unretrieved $\text{Magel2}^{+/p}$ (Fig. 1I).
Cool thermo-sensory behavior impairment in Magel2+/− neonates is not linked to a deficiency in non-shivering thermogenesis

We first supposed that this neonatal atypical thermo-sensory response could be linked to non-shivering thermogenesis dysregulation involving peripheral thermoreceptors, namely TRPM8, expressed in sensory neurons of the DRG [42]. Magel2 being expressed in the DRG [43, 44], we first analyzed whether peripheral expression of TRPM8 could be affected in Magel2+/− compared to WT neonates. We found no significant difference of the quantity of TRPM8 transcripts in DRG neurons (Fig. 2A).

We found that Magel2 is expressed in BAT (Fig. 2B) and that P2 Magel2+/− neonates had significant decreased interscapular BAT mass compared to age-matched WT (Fig. 2C). WT BAT mass was developmentally reduced from P2 to P6. Such decline was not observed in mutants. Thus, at P6, Magel2+/− neonates had significant increased interscapular BAT mass compared to age-matched WT (Fig. 2C). The difference in the amount of BAT could suggest thermogenesis dysregulation, but previous study has demonstrated that acute non-shivering thermogenesis is independent of BAT mass [45]. Thus, in order to measure non-shivering thermogenesis activity, we extracted P2 interscapular BAT tissues after 1 h exposure to cool temperature (17 °C) and analyzed BAT lipolysis and expression of the uncoupling protein 1 (UCP1), a mitochondrial protein activity marker from BAT responsible for non-shivering thermogenesis [46]. Quantitative analyses of BAT lipids, separated by thin layer chromatography (Fig. 2D), show that cold exposure induced a similar significant consumption of triglycerides (TAG) in both WT and Magel2+/− pups (Fig. 2E). Furthermore, we found that upon acute cool exposure (17 °C, 1 h), UCP1 protein expression significantly increases in P2 WT (Fig. 2F, G) as well as Magel2+/− (Fig. 2H, I). Similar results were observed at P6 (Fig. 2J–M).

Thus, these results demonstrate that UCP1-mediated non-shivering thermogenesis in BAT is fully active in Magel2+/−. They are also consistent with recent findings showing that UCP1 activation is independent of BAT mass [45]. We finally followed skin body temperatures upon cool temperature challenge and found that temperature of Magel2 deficient neonates drop similarly as WT (Fig. 2N).

Altogether, our results demonstrate that lack of pup calls reactivity to cool sensory stimuli found in Magel2 deficient neonates is related to their capacity of regulating temperature.

Cool thermo-sensory behavior impairment in Magel2+/− neonates is not linked to dysfunction of the peripheral thermosensory neurons from the Grueneberg ganglion

Peripheral perception to cool temperature is also conducted by the Grueneberg Ganglion (GG), a sensory organ located at the tip of the nose (Fig. 3A). This ganglion contains sensitive neurons responding to cool temperatures [18] and it has been proposed to influence USV [20] generated by rodent neonates to elicit maternal care under cool temperature exposure [11, 13, 21]. Interestingly, neonate mice deleted for the thermoreceptor expressed in these sensory neurons present USV calls impairment after cool exposure; a phenotype very similar to what we observe here in Magel2+/− [17]. We thus ask whether dysfunction of these peripheral thermosensory neurons might be affected in Magel2+/−. We conducted 2-photon calcium imaging and single cell analyses on tissue slices through the GG of P2 neonates (Fig. 3B, C). Thermo-evoked neuronal activities (obtained by decreasing the temperature of the perfusion solution from 35 °C to 17 °C) elicited a substantial increase in intracellular Ca2+ in both all WT and Magel2+/− animals tested (Fig. 3D, E).

Thus, the thermosensory neurons of the GG are functional in the Magel2+/− neonates.
Intranasal injection of oxytocin and oxytocin receptor agonists rescue cool sensitivity call behavior in Magel2−/−p neonates and potentiates maternal pup retrieval

We ask whether pharmacological OT treatment could improve thermosensory call behavior in Magel2−/−p during the neonatal period (P2). Of the two preferred routes to reach the cerebrospinal fluid, and considering the small size of neonate mice, we found more appropriate to administrate OT by intranasal (IN) rather than intravenous route [50, 51]. New cohorts of neonatal mice were tested for cool thermosensory call behavior with a similar procedure except that those neonates received the treatment between ambient and cool exposures. This procedure allows us to analyze the effect of an acute OT treatment by performing paired comparison between ambient versus cool exposure responses within a same animal (Fig. 5A).

We first verified that handling and IN administration procedures did not affect cool-induced call behavior of Magel2−/−p neonates. After vehicle treatment (saline solution), Magel2−/−p were still unable to react to cool exposure since the latency to the first call under cool exposure was similar to ambient exposure (Fig. 5B). Furthermore, comparison of the responsive rate to cool temperature (i.e. the proportion of neonates responsive to cooling) under cool exposure revealed an insignificant change between Magel2−/−p untreated and vehicle treated groups (Fig. 5C).

We found that IN administration of OT (2 µg) significantly decreased the latency of the first call of Magel2−/−p neonates under cool exposure (Supplemental Fig. 4A) and markedly increased the responsive rate of Magel2−/−p (Supplemental Fig. 4B). Indeed, after OT injection, 77% of Magel2−/−p neonates reacted to cool stimuli ((Supplemental Fig. 4B), a percentage...
similar to the P2 WT (Fig. 1C). Thus, an OT pharmacological treatment can rescue the cool thermosensory call behavior deficit of the Magel2+/− neonates.

To better characterize the pathway implicated in the rescue of the cool-induced call behavior, we tested two OT receptor agonists: vasopressin (AVP) which activate the OT receptor with the same affinity as OT and [Thr⁴,Gly⁷]OT also referred to as TGOT, a selective OT receptor agonist [52, 53]. We thus treated Magel2+/− neonates (P2) with either TGOT or AVP and performed USV call recording 10 min after drug administration. By analyzing the reactivity of the animals to sense cool temperature, we found that Magel2+/− neonates (P2) presented a significantly faster reaction in emitting their first call when exposed to cool versus ambient temperature after either TGOT (Fig. 5B); the difference between treatment (+Veh versus +TGOT) under cool exposure being also significant (Fig. 1B). Similar results were obtained with AVP treatment (Supplemental Fig. 4C). Furthermore, the responsive rate of Magel2+/− neonates to cool temperature was markedly increased in both TGOT (Fig. 5C) and AVP conditions (Supplemental Fig. 4D); reaching similar values as the P2 WT (Fig. 1C). We also addressed the action of TGOT in WT neonates and found that treatment preserved both the response and the number of USV upon cool temperature exposure (Supplemental Fig. 4F, H).

Finally, Magel2+/− pups treated either with AVP or TGOT evoked substantial USV call number upon cool exposure reaching values similar to P2 WT (Supplemental Fig. 4G). Although we cannot
Fig. 4 Coolness reaction failure in WT after oxytocinergic neurons inactivation. A Generation of the hM4Di DREADD OTcre mice (OThM4Di). B Immunohistochemistry illustrating the expression of hM3Di (red) in OT neurons (green). C Experimental procedure: IP injection of CNO (1 μg) or vehicle was performed in two independent groups of P2 OThM4Di neonates 2 h before starting experiment. After room habituation, neonates are separated from the dam, placed on a heating pad and each neonate is isolated for USVs recording at 25 °C for 5 min. This procedure is repeated a second time at 17 °C exposure, thus values are matched within-subject factor of “temperature”. D Before/after graphs illustrating the latency to the first vocalization upon exposure at 25 °C (red dots) followed by 17 °C (blue diamonds) in neonates expressing the hM4Di receptor (OThM4Di) treated with vehicle (Veh) or with CNO. Veh 25 °C: 3.39 ± 0.3; n = 7 vs Veh 17 °C: 0.78 ± 0.32; p = 0.07; p = 0.001. CNO 25 °C: 2.53 ± 0.48; n = 7 vs CNO 17 °C: 2.11 ± 0.60; n = 7; p = 0.04. Veh 25 °C: 3.39 ± 0.3; n = 7 vs CNO 25 °C: 2.53 ± 0.48; n = 7; p = 0.08. Repeated-measures (temperature) Two-way ANOVA, Bonferroni’s post-test. E Responsive rate of coolness-induced USV in OThM4Di neonates treated with vehicle or CNO (85.71 ± 14.29%, n = 7 vs 36.36 ± 15.21%, n = 11; p < 0.0001). Cooling reactivity of OThM4Di neonates treated with CNO was also compared with either Magel2+/−, (37.5 ± 12.5%, n = 16; p = 0.1169) or neonates non-expressing the hM4Di receptor (hM4Di) (50 ± 16.67 ln-1 s−1, n = 10, p = 0.0315). Fischer’s exact test. F, G Total number of calls at 25 °C (F) and 17 °C (G) in OThM4Di neonates treated or not with CNO and compared with either Magel2+/− or neonates non-expressing the hM4Di receptor (hM4Di). F OThM4Di (veh vs CNO): 205 ± 45, n = 8 vs 105 ± 44, n = 13; p = 0.5. OThM4Di+CNO: 105 ± 44, n = 13 vs hM4Di+CNO: 245 ± 39, n = 12; p = 0.07. G OThM4Di (veh vs CNO): 134 ± 28, n = 8 vs 72 ± 32, n = 13; p = 0.18. OThM4Di+CNO: 72 ± 32, n = 13 vs Magel2+/−: 111 ± 24, n = 16; p = 0.53. OThM4Di+CNO: 72 ± 32, n = 13 vs hM4Di+CNO: 111 ± 20, n = 12; p = 0.52. Kruskal-Wallis test, Dunn’s post-test. Data are presented as mean ± SEM.

completely exclude a minor contribution of the AVP receptors, these data suggest that the improvement of cool thermosensory call behavior is mainly due to activation of the OT receptors.

Since cool exposure or stress alters the Erk pathways in the brain by reducing Erk activation [54, 55] and OT has been shown to block this alteration [54], we explored whether these signaling pathways might be altered in Magel2+/− brain. Cytoplasmic levels of P-Erk were measured from P2 whole brains of WT and Magel2+/− immediately after ambient or cool exposure. Cytoplasmic levels of P-Erk revealed that brain of WT neonates had a significant cool-induced reduction of P-Erk (Supplemental Fig. 5A, C); while Magel2+/− did not (Supplemental Fig. 5B, D). More importantly, inactivation of OT allowed a cool-induced reduction of P-Erk in the Magel2+/− neonates (Supplemental Fig. 5F, H) without affecting the reduction of P-Erk in the brain of WT (Supplemental Fig. 5E, G). Thus, these results highlight a deficit in Magel2+/− brain development and reveal that OT’s ability to reverse cool thermosensory call behavior may act, at least partly, through Erk pathways.

Considering these results, the fact that OT of mother’s brain is an important modulator of the mother-infant bond [33, 56] and that Magel2+/− pups present a dysregulation of the oxytocinergic system [31, 32], we hypothesized that OT of pup’s brain could be also implicated in this bound. By treating Magel2+/− pups with TGT (0.2 μg, IN), we found an improvement of dam’s performance to retrieve Magel2+/− pups under cool exposure compared to ambient condition (Fig. 5E): as observed in WT pups (Fig. 1G). This result was not observed in vehicle-treated Magel2+/− pups (Fig. 5E). By measuring every 30 s throughout the test the proportion of retrieved pups, we found that while only 7% of vehicle-treated Magel2+/− pups were retrieved, more than 70% of them were retrieved after TGT treatment (Fig. 5F). At the end of the test, the proportion of unretrieved Magel2+/− pups was also significantly reduced by TGT treatment since all pups were retrieved under cool condition (Fig. 5F). Finally, on analyzing the effect of TGT treatment under ambient condition, we observed that during the first 30 s the proportion of retrieved pups was around 15%, while at the end of the test all TGT-treated Magel2+/− were retrieved (Fig. 5G).

Thus, these results demonstrate that maternal pup retrieval is enhanced after administration of the OT receptor agonist in Magel2+/−.

DISCUSSION

ASD research has mainly focused on ASD-related genes and their impact on social and cognitive behavior in adult. However, atypical sensory reactivity that represents early markers of autism and are predictive of social-communication deficits and repetitive behaviors in childhood has been largely overlooked. Although recent findings performed in mouse ASD genetic models report sensory deficits [2–8], they were explored during juvenile or adult period. Whether sensory dysfunctions might be present at the early life stage is still unknown.

Here we provide the first experimental evidence that newborn harboring deletion in Magel2, an autism associated-gene implicated in Prader-Willi and Schaaf-Yang syndromes, exhibit atypical thermosensory behavior during the first postnatal week.

With the aim to investigate any impairment in physiological thermogenesis, we found BAT activation upon cool challenge, suggesting that the autonomic neural circuit controlling non-shivering thermogenesis is not affected and cannot be incriminated for this atypical thermosensory behavior.

Furthermore, functional investigation of the GG revealed that this peripheral cool sensor is still active in Magel2+/− neonates. Magel2+/− neonates might encounter difficulties in integrating thermo-sensory stimuli and we provided some evidence for such deficits. First, we can rule out a motor/vocalization deficit since USVs calls were unaffected under ambient temperature at this early developmental stage. We found a lack of cool-induced alteration of brain P-Erk signaling. Second, we showed that brain inactivation of OT neurons in WT reproduces atypical thermosensory reactivity. Third, intranasal injection of OT or its agonists can improve thermo-sensory reactivity through activation of the OT receptor. Whilst it is well known that OT plays a major role in modulation of social and cognitive behavior in adult. However, adolescent with ASD present loss of sensory function of life and after an indicator of an affective state.

This hypo-thermosensory reactivity of the oxytocinergic system in modulating early life thermosensory function that could be involved in these symptoms.

This hypo-thermosensory reactivity of the Magel2+/− neonates to cool environment is rephrased on the reactivity of the mother to retrieve their pups. We have demonstrated the existence of a substantial proportion of unretrieved Magel2+/− pups by their WT dams as well as a delayed latency to retrieve the rest of them under cool exposure. Deficit in maternal care of Magel2+/− has been reported recently to occur specifically at P8 but not P6 [57]. Whilst they used a different Magel2 mutant mouse model (Magel2<sup>2mi15Sw</sup>) and a distinct experimental approach, the difference in age is also an important factor. Therefore, it is possible that maternal care evolves during age pup being more an indicator of danger (here temperature drop) during the first week of life and after an indicator of an affective state.

OT agonist treatment of Magel2+/− neonates improved both the proportion and the latency to retrieve. This suggests that the
oxytocinergic system at P2 modulates the reactivity of newborns to rapidly generate USVs which has a direct effect on mother’s behavior. OT and USVs calls play an important role in mother-infant recognition [29, 58] and they are both dysregulated in this mouse autistic model. Our present results highlight that not only OT from the dam, but also OT from the pups is important in this mother-infant recognition.

Although cool-induced cry has been also observed in newborn infants more than 20 years ago [59], it is rarely observed nowadays because maintaining the body temperature of the neonates has
been emphasized. Atypical early vocalization has been detected in 6-month-old infants at risk for autism [14] and may represent an early biomarker for ASD [15]. Measures of early life sensory behavior such as cool-thermosensory call behavior might represent promising avenues for early diagnosis and OT treatment could be considered for therapeutic interventions of this atypical sensory reactivity.

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