Lightweight, wireless LED implant for chronic manipulation in vivo of spontaneous activity in neonatal mice

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ABSTRACT

Background: Long-term manipulation of activity in the neonatal rodent brain can help us understand healthy development, but also involves a set of challenges unique to the neonatal animal. As pups are small, cannot be separated from their mother for long periods of time, and must be housed in a nest, many traditional techniques are unusable during the first two postnatal weeks.

New method: Here, we describe the use of magnetic resonance induction to allow wireless and chronic optogenetic manipulation of spontaneous activity in mouse pups during the second postnatal week.

Results: Pups were implanted with a lightweight receiver coupled to an LED and successfully returned to the homecage. A transmitter coil surrounding the homecage drove the implanted LED and was regulated by a microcontroller to allow flexible, precisely-timed and wireless control over neuronal manipulation. In vivo patch-clamp recordings verified that activation of the LED triggered bursts of action potentials in layer 2/3 neurons that expressed channelrhodopsin-2 in the visual cortex without directly affecting neighboring, non-expressing neurons. The implants are stable and functional for at least 10 days and do not have an impact on the weight gain of pups. Implanted pups’ behavior is mildly affected only briefly after surgery, while maternal behavior of dams remains unaffected.

Comparison with existing method(s): In contrast to most other methods for wireless optogenetic stimulation, the small size and low weight of the receiver allow complete implantation in animals that are as small as a newborn mouse.

Conclusions: This method is ideal for investigating the function and development of cortical circuits in small and developing animals. Furthermore, our method is economical and easy to adapt to diverse experimental designs.

1. Introduction

During early development, the connections between young neurons are refined into functionally useful networks in an activity-dependent manner. Bursts of spontaneously generated activity travel through the brain, pruning unwanted synapses and strengthening well-targeted connections (Luhmann et al., 2016; Simon and O’Leary, 1992). Understanding how spontaneous activity controls these crucial choices teaches us about the fundamental processes that underlie the formation of the brain, and may also help us understand and treat neurodevelopmental disorders (Cheyne et al., 2019; Goncalves et al., 2013).

Experimental manipulation of spontaneous activity is one of the most direct and powerful ways to test the consequences of activity patterns on the network. However, attempting to manipulate activity in very young rodents comes with unique challenges. Firstly, neonatal animals are too small for conventional methods that require heavy head plates or bat-teries. At ten days old a mouse will weigh around 6 g (Greenham, 1977), preventing use of most modern headstages. Secondly, repeatedly...
removing pups from the mother is a stressful experience that can have consequences for neural and behavioral development in a manner that is difficult to predict (Orso et al., 2019; Tractenberg et al., 2016) and is therefore best avoided if possible.

Chemogenetic manipulations are one possibility (Urban and Roth, 2015), and can be performed with one or two daily injections of the agonist, preventing long absences from the mother. However, chemogenetic manipulations are best suited to applications where a blanket change is required, such as an overall reduction in signaling from one neuron type or a specific subset. Spontaneous activity is carefully patterned; the frequency, participation and synchrony of the activity are not random characteristics, but contain information that give spontaneous activity the ability to shape the network (Kirikby et al., 2013; Leighton and Lohmann, 2016). Therefore, mimicking or manipulating this activity would ideally involve specifically timed changes in firing. To circumvent these difficulties, we describe here an extremely light-weight, wireless optogenetic tool, which allows precise and reliable induction of spikes in pups between postnatal days (P) 8 – 14. To our knowledge, this is the first description of a method that allows pups to return to the nest environment while receiving induction of neuronal activity.

2. Methods

2.1. Animals

All experimental procedures were approved by the institutional animal care and use committee of the Royal Netherlands Academy of Sciences. Mice of both sexes were used. All animals were aged between postnatal days (P) 8–20. Wild-type mice were either C57BL/6J mice or C57BL/6J × CBA F1. These mice open their eyes at P14.

2.2. In utero electroporation

Pyramidal neurons in layer 2/3 of the visual cortex were transfected with plasmid DNA driving expression of channelrhodopsin-2 (ChR2) (2 mg/ml, pCAGGS-ChR2-Venus; Addgene plasmid 15753, Petreanu et al., 2007) and DsRed (2 mg/ml, pCAGGS-DsRed; a gift from Christiaan Levelt) at E16.5 using in utero electroporation (Harvey et al., 2009). Pregnant mice were anesthetized with isoflurane and a small incision (1.5–2 cm) was made in the abdominal wall. The uterine horns were carefully removed from the abdomen, and DNA was injected into the lateral ventricle of embryos using a sharp glass electrode. Using an electroporator (ECM 830, BTX), voltage pulses were delivered across the brain with tweezer electrodes covered in conductive gel (five square waves, 30 V, 50 ms duration, 950 ms interval). Embryos were rinsed with warm saline solution and returned to the abdomen, after which the muscle and skin were sutured.

2.3. Whole-cell recordings

Animals aged P9-P12 were mildly anaesthetized (0.7–1% isoflurane at 1 L/min) during in vivo whole-cell recordings. Membrane potential was recorded in current clamp at 10 kHz and low pass filtered at 3 kHz (Multiclamp 700b, Molecular Devices) as described previously (Leighton et al., 2021). For current clamp recordings, electrodes (4.5–6 MΩ) were filled with intracellular solution (105 mM K gluconate, 10 mM HEPES, 30 mM KCl, 10 mM phosphocreatine, 4 mM MgATP, and 0.3 mM GTP; Golshani et al., 2009). An LED-receiver with long wires was assembled to facilitate opticogenetic stimulation during in vivo whole-cell recordings. This allowed placement of the wirelessly powered LED at the electrophysiology setup while the receiver coil remained within the induction coil inside the Faraday cage. A piece of skull was attached to the LED with super glue to avoid direct illumination through the cranial window, and the LED was placed as close as possible to the recording site to achieve the highest possible power for stimulation. LED optical output power was measured at 473 nm, using a power meter console (PM100A, Thorlabs) combined with an integrating sphere photodiode power sensor (S142C, Thorlabs).

2.4. Pup body weight tracking

Pups’ weight was measured for up to 10 days after implant surgery. Pups were individually removed from the nest, placed on a tray for measurement on a compact precision balance (KERN 440–47 N) and immediately returned to the nest. On the day of surgery, the weight of implanted pups was measured before surgery and after recovery from anesthesia. The difference between post- and pre-surgery weight was subtracted from their follow-up weight measurements for comparison with littermates.

2.5. Maternal behavior assessment

The homecage was placed inside the Faraday cage, on the polyethylene housing, right above the transmitter coil. The interlock switch was deactivated and the Faraday cage was kept open to allow acquisition of overhead video recordings through the mouse cage lid using a Raspberry Pi controlled camera module. Homecage video recordings of 30 min duration were acquired twice daily, starting the day after implant surgery. Each day, a recording started 30 min before the wireless system was turned on (LED-off period), and another recording began 30 min before the wireless system was turned off (LED-on period). The wireless system was turned on for 2 h during the light phase, in the morning or afternoon alternately. These periods consisted of 1-second duration light pulses with a frequency of 2 pulses/minute. Maternal behavior in the homecage was quantified as duration of dam presence within the nest in contact with pups (total time in nest), and number of times that the dam left the nest (number of nest exits). These parameters were analyzed for each observation session to allow comparison of maternal behavior during LED-on vs. LED-off periods.

2.6. Pup open field test

Pups were implanted at P10. Operated pups and littermates were tested on day 2 after surgery and every 48 h thereafter. Animals were individually placed in the central area of a square open field measuring 25 cm along each side, and the area was recorded for 6 min. The bottom of the open field was heated at approximately 30 °C and covered by an absorbent bench pad, which was replaced after each trial. A Raspberry Pi controlled camera module was used to capture video recordings at a frame rate of 25 Hz. Animal tracking was performed using a custom-made workflow in Bonsai (Lopes et al., 2015). Pups’ centroid coordinates were processed with a custom-made Python script to obtain average speed, maximum speed, total distance traveled and total time in the center of the open field (a concentric square of 12.5 cm side). Only periods with speed > 0.5 cm/s were included to compute the average speed during locomotion.

2.7. Data analysis

We used R (R Core Team, 2021) to fit linear mixed-effects models (LMMs) with the lme4 package (Bates et al., 2015). We evaluated the statistical significance of fixed effect terms in each model using the ANOVA test of the lmerTest package (Kuznetsova et al., 2017), with Satterthwaite’s method for computation of denominator degrees of freedom and F-statistic. Linear mixed-effects models are represented as population-level mean and 95% confidence intervals. In all cases, x-axis jitter was applied to improve visualization of single data points.

We constructed an LMM to evaluate the effect of the LED implant on pups’ weight over postnatal days. We used group (operated pup or littermate), age and group-age interaction as fixed effects. Litter and pup were included as nested random effects.
LMMs were also fitted to evaluate maternal behavior. We constructed an LMM for each maternal behavior measurement (total time in nest and number of nest exits). Both models included condition (LED-on or LED-off), days after surgery and time of day (AM or PM) as fixed effects, and dam as a random effect. Considering that neither days after surgery nor time of day had a significant effect on the total time in nest or number of nest exits, we pooled the data of all recording sessions in each condition for each dam and performed Wilcoxon signed-rank tests of LED-on vs. LED-off conditions.

To evaluate the effect of the LED-receiver implant on pup locomotion, we fitted an LMM for each measurement evaluated (total distance, maximum speed, average moving speed and time in center). Condition (operated pup or littermate) and days after surgery were included as fixed effects, with an interaction term. Litter and pup were included as a nested random effects term.

3. Results

3.1. Wirelessly activating LED implants through resonance induction

Our experimental setup is shown in Fig. 1A and its main components are listed in Table S1. Pups are housed with their mothers in their nests inside a normal homecage. This cage is kept inside a larger Faraday cage (35 × 42 × 36 cm, Fig. S1A) with an embedded induction coil to generate the magnetic field for current induction. A custom box containing a microcontroller (Arduino UNO), sine wave generator and an amplifier allow the experimenter to turn on the induction loop and adjust the magnetic field strength inside the cage. A standard lab voltage supply powers this box. Driving the system using an open-source microcontroller allows the user to easily adjust the duration and pattern of stimulation using a custom-made script (Supplemental File 1). The field is resonant at 6.78 MHz.

The Faraday cage is equipped with an interlock switch, such that the voltage on the transmitter coil is automatically stopped when the cage lid is open for animal care (Fig. S1B). As an additional safety measure, the transmitter coil is encapsulated in a waterproof polyethylene box so that all potentially high voltages are strictly inside this plastic housing.

Target pups are implanted with a receiver coil coupled to an LED, which receives a current when the induction loop is active (Fig. 1B). The LED-receiver implant weighs approximately 80 mg (Fig. 1C) and it can be easily assembled (Fig. S2). It contains only a small capacitor (1.6 × 0.8 × 0.8 mm), a copper coil printed on malleable flex circuit board, and a surface mounted super bright blue LED (1.6 × 0.8 × 0.6 mm). Heating the implant around a cylindrical mould allows it to conform to the shape of the pup’s back. We covered the flex board with silicone coating to protect the implanted components and smooth out any rough edges. Depending on the experimental goal, various wavelengths or types of LED could be used on this implant.

3.2. Verifying neuronal activation

During a burst of spontaneous activity, many neurons are active simultaneously, firing a few action potentials in a row before falling silent again (Colonnese et al., 2010; Rochefort et al., 2009). As spontaneous activity most likely relies on burst-dependent synaptic plasticity to wire up the developing brain (Butts et al., 2007; Butts and Rokhsar, 2001), we aimed to mimic patterns of spontaneous activity during the second postnatal week.

Our chronic manipulation approach takes advantage of the thin skull of neonatal rodents to provide the light stimulation through the skull.
rather than performing a craniotomy. Above 80% of the blue light emitted by the LED is transmitted through pups' skull at P16 (83.4 ± 4.5%; mean ± SD, n = 8 parietal bones). To test that the light intensity of the LED was sufficient to activate cells even through the skull, we performed in vivo whole-cell recordings. We expressed the light-sensitive channel ChR2 in pyramidal cells in visual cortex layer 2/3 (L2/3) by performing in utero electroporation at E16. The red fluorescent protein dsRed was co-expressed for easy recognition of cells (Fig. 2A). We used two-photon-targeted whole-cell recordings of pyramidal cells in vivo to record spiking responses to illumination by the LED through the neonatal skull. Cells expressing ChR2 responded reliably to the light stimulus (Fig. 2B-D). With 1 s duration of stimulation at 5 mW LED optical output power, we obtained burst patterns similar to natural spontaneous activity (Fig. 2C). The spiking frequency of cells that did not express ChR2 was not affected by LED activation (Fig. 2E).

3.3. Optimizing implant protocol for pup re-acceptance

The next step was to find a suitable protocol for implantation (Fig. 3). The LED-receiver must be implanted in a manner that does not prevent the dam from accepting operated pups back into the nest. Dams can reject or kill pups that appear weak or harmed, so a smooth surgery is essential.

3.3.1. Wireless implant

3.3.1.1. Equipment.

- LED-receiver implant
- Curved forceps (Item 11652–10, Fine Science Tools)
- Surgical scissors (Item 14084–08, Fine Science Tools)
- Ring forceps (Item 11106–09, Fine Science Tools)
- Probe (Item 10140–01, Fine Science Tools)
- Blunt needle or microhook (Item 10062–12, Fine Science Tools)
- Compact mouse stereotaxic instrument (Base plate and ear bars, Harvard Apparatus)
- Microbrushes (Superfine or ultrafine, Microbrush)
- UV LED curing light (Premium Plus)
- Fluorescence detection lamp
- Heating pad
- Pup-sized isoflurane nose cone

3.3.1.2. Reagents.

- Dental bonding agent (iBond Universal, Kulzer)
- Dental cement (Charisma Flow, Kulzer)

Fig. 2. LED-mediated in vivo neuronal activation of ChR2-expressing cells, (A) Cells expressing dsRed in cytoplasm and ChR2-Venus in membranes. (B) Example in vivo whole-cell recordings in cells with (above) and without (below) ChR2. (C) Action potentials fired during optogenetic stimulation. Recorded in whole-cell configuration in cells with (above) and without (below) ChR2. (D) Frequency of action potential firing before (grey) and during light stimulation (blue) in two cells expressing ChR2. (E) Frequency of action potential firing before (grey) and during light stimulation (blue) in two cells not expressing ChR2.
3.3.1.3. Protocol.

1. Remove the pup from the nest using gloves.

2. Weigh to establish baseline body weight.

3. Induce isoflurane anaesthesia at 3% for 3 min in an induction box.

4. Remove and place pup on a heating pad in custom, pup-size nose cone and hold in place with ear bars.

5. Scrub the body of the pup gently with 70% (vol/vol) ethanol. Inject meloxicam subcutaneously into flank (0.025 ml, approx. 0.4 mg/kg).

6. Reduce isoflurane to 2%. Check approximate position of LED-receiver implant.

7. Using scissors, make a straight opening in the back of the pup for the receiver coil. To facilitate the insertion of the receiver coil, lift the skin on each side of the incision and use a probe to gently separate the skin.

8. Cut a very small opening on the side of the head that expresses the construct.

9. Using a fluorescence detection lamp, verify the exact location of expression in the cortex.

10. Clean the target area with sterile saline solution and apply dental bonding agent on the skull, cure it with UV light.

11. Use a blunt needle or microhook to create space to tunnel the LED under the skin, from the back to the head.

12. Remove the tool and use it to guide the LED and wires through the space created.

- Skin glue (3M Vetbond Tissue Adhesive)
- Super glue (Bison secondelijm)
- Anti-nail biting liquid (Byte-X, denatonium benzoate solution)
- Local analgesic (Meloxicam 0.08 mg/ml, injectable solution)
- Ethanol 70% (vol/vol)

Fig. 3. Surgical procedure for LED-receiver implantation, (A) Pup is placed on the surgical setup (steps 1–5). (B) LED-receiver is held on top of the animal to estimate incision sites (step 6). (C) Skin incisions are made on the back and head of the animal using surgical scissors and forceps (steps 7 and 8). (D) Dental bonding agent is applied on the skull over the target area (steps 9 and 10). (E) A blunt needle or microhook is inserted to create space to tunnel the LED subcutaneously (step 11). (F) LED and wires are tunnelled from the back to the head of the animal (step 12). (G) Receiver coil is inserted, making sure LED and wires are lying flush with the body of the animal (steps 13 and 14). (H) LED is attached to the skull with super glue (step 15). (I) Dental cement is applied on top of the LED, covering all visible skull (step 16). (J) Back incision is closed with skin glue (step 17). (K) Anti-nail biting liquid is applied around both incisions (step 18).
13. Shape the receiver wires with forceps so that the LED will naturally fall down on the skull.

14. Insert the coil and verify that the receiver lies flush with the body of the animal.

15. Apply super glue to LED and glue to skull.

16. Cover the LED and exposed skull with a layer of dental cement, and cure it with UV light. Do not attempt to close the opening on the head as this increases the chance of the mother removing the implant. Instead, make sure the dental cement covers all exposed skull.

17. Close the opening on the back with small dots of skin glue.

18. Apply anti-nail biting liquid around both incisions to prevent the mother from overgrooming the wounds. Apply this liquid on the same areas of non-implanted littermates to avoid differential maternal behavior due to pups’ smell or taste.

19. Turn off isoflurane and allow pup to wake up. Weigh the pup again to establish post-implantation body weight. Ensure that the pup is fully warm before returning to the nest.

3.4. Studying the consequences of LED-receiver implants and wireless LED activation

We found that the protocol described above resulted in pups being accepted by the mother: the implanted pups were returned to the nest and fed (Fig. 4A); the implant remained in place and functional for up to 10 days after surgery (Fig. 4B); and the implanted pups’ weight was not different from their non-implanted littermates (Fig. 4C) and similar to previously reported C57BL/6 mice pre-weaning weight (Castelhano-Carlos et al., 2010; Spangenberg et al., 2014). All operated pups survived the surgical procedure and were returned to the nest. A total of 20 pups from 9 litters were operated, of which 11 pups from 7 litters were successfully implanted (Table S2). We considered pups were successfully implanted if the LED-receiver remained in place and functional for a minimum of 48 h after surgery.

To test if wireless stimulation may induce changes in maternal behavior, we observed dams’ activity in the homecage twice daily during LED-on and LED-off periods (Fig. 5A). We quantified each dams’ time spent in the nest in contact with pups and number of nest exits, and found no difference between LED-on vs. LED-off conditions (Fig. 5B-C).

The behavior of implanted pups and non-implanted littermates was evaluated every 48 h after surgery with open field testing. We observed pups’ open field trajectories (Fig. 5D) and measured their locomotion as total distance traveled, maximum speed and average moving speed. Considering that initial testing was performed at P12 and thigmotaxis emerges before eye opening (Bass et al., 2020), we also measured the time that pups spent in the center of the open field. The LED-receiver implant had no significant effect on the total distance, maximum speed or average moving speed achieved by pups during the open field test (Fig. 5E-G). We found a difference between operated pups and littermates regarding the time spent at the center of the open field (Fig. 5H), but this difference was only present two days after surgery and was due to the shorter time that implanted pups took to initially find the border of the open field arena instead of reflecting different exploratory behavior.

Fig. 4. Re-acceptance of implanted pups into the nest, (A) Pups were accepted back into the nest after surgery. White arrow: dental cement cap. (B) Implanted LEDs remained functional after operated pups were returned to the nest. (C) The LED-receiver implant has no significant effect on pups’ weight over postnatal days, \( F(1, 225.52) = 1.13, p = .29 \). Linear mixed-effects model and single pups’ weight measurements are shown (16 operated pups and 30 littermates, 7 litters).

4. Discussion

Here, we describe an approach for wireless activation that allows young pups to remain in the nest with their mother while receiving specifically timed optogenetic stimulation.

There is a range of different possible methods for wireless optogenetic stimulation, reviewed in Qazi et al. (2018). Head-mounted devices, at around 2–3 g, cannot be supported by the pups at this age. Use of, for instance, an aimed laser is impractical when housing the pups in the homecage with the mother (Wang et al., 2020). Instead, we used a resonance induction method similar to Shin et al. (2017) and extended the application of wireless optogenetics to pups during the second postnatal week. We optimized an implantation surgical procedure which allowed pups to recover well, be taken back into the nest and cared for by the dams. Our method provides light stimulation through the thin skull of neonatal mice, which limits the optogenetic manipulation to superficial layers due to a quick drop of blue light power transmission as
a function of distance from the light source in brain tissue (Aravanis et al., 2007). A possibility would be to combine a fiber-coupled LED similar to the one presented by Lee et al. (2015) with our wireless approach to allow stimulation of deeper brain areas while adding relatively little weight, although it would require a more invasive surgical procedure.

The LED-receiver implant had a minor impact on pups’ behavior only briefly after surgery, and it did not have an effect on pups’ weight gain. LED illumination did not affect maternal behavior. Our stimulation protocol was carried out during the light phase, and blue LED light exposure during daytime has not been linked to disruption of maternal behavior or offspring brain development in mice (Horibe et al., 2017). To carry out a stimulation protocol during the dark phase, we recommend the use of black dental cement during the surgical procedure to limit LED illumination through the dental cement cap, as exposing mice to light at night has substantial influences on their physiology and behavior (Emmer et al., 2018).

Safety of both investigators and experimental animals is an important concern in building setups with high voltages and magnetic fields. The operating frequency of 6.78 MHz, within the industrial, scientific and medical purposes (ISM) radio band, was chosen to prevent interference of the magnetic field with other equipment. This frequency is much lower than the 2.45 GHz frequency that induces heating in a microwave oven, and it presents a negligible heating effect of metallic objects compared to lower frequency wireless power transfer alternatives (Tseng et al., 2013). There is no notable absorption of energy by the mouse cage, chow, water or bedding when using the stimulation settings described here. To prevent possible interference with other equipment, we placed the transmitter coil inside a Faraday cage. Putting it in a Faraday cage slightly alters the resonance frequency, but this can be tuned easily with the choice of capacitors. The expected magnetic field strength at the center of the receiver coil equals approximately 4.4 A/m in practical use at 5 mW LED optical output power (Supplemental information 1), which is well below the occupational reference safety level.
for protection of humans exposed to time-varying electromagnetic fields (ICNIRP, 2020, 2010). The current induced in the mouse brain is limited by the impedance of tissue inside the brain. We compared the magnetic flux density of our system to typical transcranial magnetic stimulation (TMS) threshold levels, usually above 1 T, and to subthreshold TMS levels which have been shown to modulate neuronal plasticity or have an effect on behavior, as low as a few mT (Lloyd et al., 2020; Malekiewicz et al., 2014; Rodger et al., 2012; Tang et al., 2018, 2021). Our wireless system operates at a level of 5.5 µT, a thousand times below subthreshold TMS intensities and over 150 thousand times below TMS threshold.

While using this technique, it is important to consider that the developmental stage at which the optogenetic stimulation is provided may have an impact on the effect observed. Optogenetic stimulation of ChR2 positive L2/3 pyramidal neurons drives inhibition of other L2/3 pyramidal cells at adult age (Adesnik, 2018; Chettih and Harvey, 2019), while this effect was absent at P9-P12 likely as a consequence of immature intracortical connectivity at this age (Chevée and Brown, 2018; Hooks and Chen, 2020). The repercussion of evoking additional activity for the whole network should also be taken into account, as it is possible that evoked activity suppresses intrinsic activity, rather than simply occurring side-by-side. Acute experiments using, for instance, calcium imaging, could confirm whether wirelessly induced spontaneous activity occurs in a truly additive manner, or whether the developing network adapts to the manipulation. We look forward to further technological advancements that may allow our wireless optogenetics approach to be combined with spontaneous activity monitoring to develop a closed-loop approach. A device capable of recording continuous EEG in immature rodents has been developed and weighs relatively little (Zayachkivsky et al., 2015), but it is at least 6 times heavier than the LED-receiver implant described here. Currently, a feasible closed-loop system could be developed by coupling the wireless stimulation to certain pup behavior, such as vocalizations.

The ability to manipulate spontaneous activity accurately, reliably and chronically in the neonatal mouse, without excessive disruption of its behavior, opens up a wide range of fresh research opportunities. This is also relevant to neurodevelopmental disease models; mice with the Fragile X (FragX) mutation have more synchronized spontaneous activity than wild-type animals (Chevée et al., 2019; Goncalves et al., 2013). By allowing specifically timed activation of neurons, the information encoded in the characteristics of spontaneous activity can be tested using this wireless system.

Compared to other long-term manipulations, one of the major benefits of this technique is that it is flexible. The choice of stimulation protocol will depend on the research question at hand, and may have different consequences depending on the developmental stage. The microcontroller allows the user to adjust the pattern of activity induced using millisecond precision, and the power supply can be adjusted to increase or decrease magnetic field strength. This is particularly relevant in pups as spontaneous activity patterns change quickly during development (Ackman et al., 2012; Colonnese et al., 2010; Rochefort et al., 2009; Siegel et al., 2012), requiring tailored stimulation paradigms to match a pup’s age. This is in contrast to, for instance, expressing a chemonogenetic construct such as DREADD, which can either excite or inhibit cells but without precise timing. Here, we used the ‘classic’ opsin ChR2, but recent years have seen the development of many new types of opsin (Deisseroth and Hegemann, 2017; Schoenenberger et al., 2011). Their different sensitivities and kinetics could be used to adapt this technique depending on the desired manipulation.

CRediT authorship contribution statement

Alexandra H. Leighton: Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Writing – original draft. M. Victoria Fernández Busch: Methodology, Formal analysis, Investigation, Visualization, Writing – original draft. Joris E. Coppens: Methodology, Resources. J. Alexander Heimel: Writing – review & editing, Supervision. Christian Lohmann: Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Conflict of interest statement

We declare no conflict of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jneumeth.2022.109548.

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