

Ultrasonic Vocalisations (USVs) in rat pups

An animal friendly marker for neurotoxicity during development

Studies to test developmental toxicity involve daily testing of hundreds of rats to reflect on their development. This behavioral survey during the pre-weaning phase requires intensive handling of the rat pups to study their physical and sensory development. Although not invasive, these activities greatly disturb normal housing and nursing of the litter in the home cage. We hypothesized that to test development, so called Ultrasonic Vocalizations (USVs) of the rat pups, i.e. calls of 35 – 65 KHz emitted by rodent pups to communicate with their mothers [1], could be an alternative animal friendly marker with high discriminative power.

RESEARCH QUESTIONS

- Are USVs a marker for normal (neural) development of rat pups?
- Are USVs a marker for developmental (neuro) toxicity?
- Can USVs be used as a fast screening tool?

METHODS

Developmental toxicity study with organotin compounds

• Di-octyl-tin-chloride (DOTC)

- In packing material
- Especially toxic for developing immune system
- Tri-butyl-tin-oxide (TBTO)
 - In marine paint
 - Especially toxic for developing neural system

Exposure

- **DOTC**
 - Diet; 2 wks pre-mating F0 to adulthood of F1 animals
 - 0, 3, 10, 30 mg DOTC/kg BW/day
- **TBTO**
 - Diet; day 6 of pregnancy to postnatal day (PND)10
 - 0, 8 mg TBTO/kg BW/day

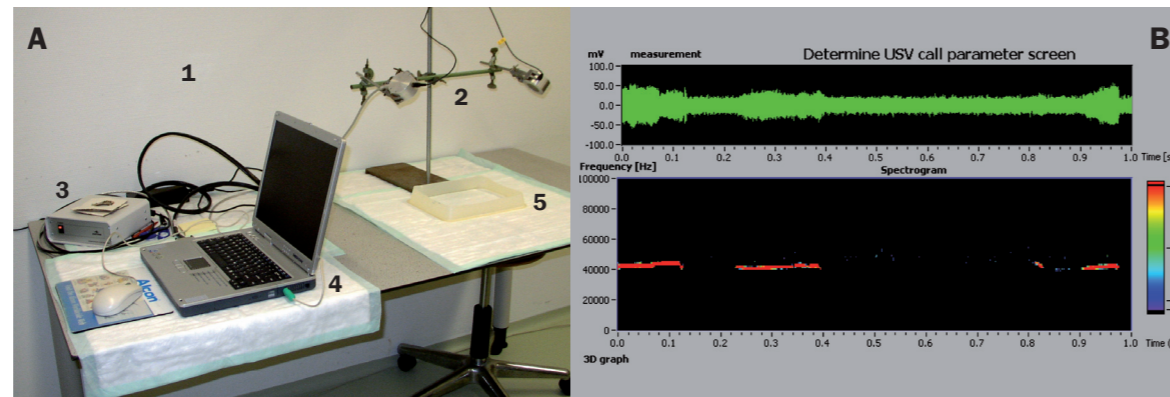


Figure 1. SonoTrack* hardware and test set-up for data acquisition of ultrasonic vocalizations (USV) in rat pups. Figure 1A: 1. Up to four channel units with two microphones (2) each; 3. SonoTrack Control Unit; 4. PC with SonoTrack software; 5. test arena for rat pups. Figure 1B: SonoTrack, Play back raw data screen. * SonoTrack was developed by Metris BV, Hoofddorp, The Netherlands in co-operation with TNO, the largest independent research organization in the Netherlands.

Data acquisition USVs [Figure 1]

- From PND 4-18
- Every morning same time
- 30s alone in open arena

Data analyses USVs

- Number of USV calls
- Frequency (Hz) of USV calls
- The data were smoothed using a penalized least squares smoothing algorithm

RESULTS

Figure 2 (number of calls)

- Controls: peak of calling around PND 11
- TBTO: maximum number of calls ↓
- TBTO: slope (later postnatal day) to maximum number of calls ↑
- TBTO: mean number of calls / treatment ↓

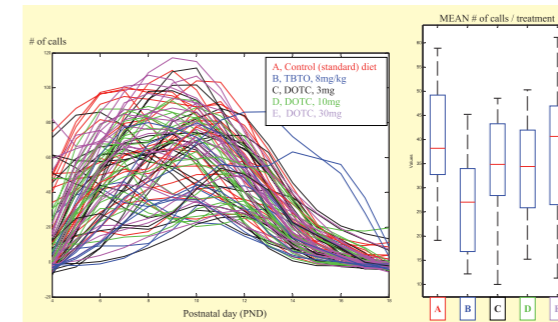


Figure 2. Ultrasonic vocalizations (USV) in rat pups (PND 4 to 18). Effects of organotin compounds DOTC (3 dose groups) and TBTO (in daily diet) on the number of USV calls per test day (left panel) and mean number of calls time per treatment (PND 4 to 18) (right panel). Under standard diet conditions, a typical pattern of development of USV calling is observed from PND 4 to 18 with a maximum of USV calling at about PND 11. TBTO reduced the maximum number of calls, which in addition was reached at a later postnatal day, resulting in different slope of the USV-curve over time (left panel) and reduction of mean number of calls/treatment (right panel). DOTC did not affect the development of the USVs.

Frequency of USV calls (Figure 3 and Figure 4)

- Controls: dip of frequency around PND 11
- TBTO: tendency to increased frequency at start (PND4) and decreased at end (PND18)
- TBTO: mean frequency / treatment ↑

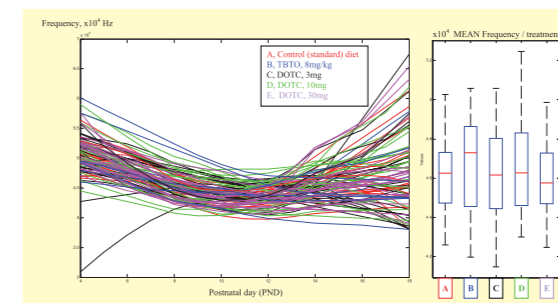


Figure 3. Ultrasonic vocalizations (USV) in rat pups (PND 4 to 18). Effects of organotin compounds DOTC (3 dose groups) and TBTO (in daily diet) on the frequency of USV calls per test day (left panel) and mean frequency per treatment (PND 4 to 18) (right panel). TBTO affected the frequency of the USV calls: tendency to increase of frequency at early days (PND 4) and reduced frequency towards PND 18 (left panel); mean frequency per treatment (PND 4 to 18) was increased (right panel). DOTC did not affect the development of the USVs. See also figure 4.

CONCLUSIONS

- Ultrasonic vocalizations in rat pups provide a marker for normal development.
- A typical distribution curve of calling exists from PND 4 to 18, with a peak around PND 11.
- DOTC, a developmental immunotoxicant, did not affect the number and frequency of the USV calls.
- TBTO, a developmental neurotoxicant, affected both the onset and development of number and frequency of the USV calls.

Together, the results point at delayed neural development in TBTO exposed rats and support the hypothesis that USVs in rat pups can act as a marker for normal neural development and developmental neurotoxicity. The results further indicate that the test conditions proposed (30 seconds alone in an open arena) warrant fast, reliable and animal friendly screening of preweaning development of rats.

REFERENCES

- [1] Branchi *et al.*, 2001, *Behav. Brain Research* 125 49–56.

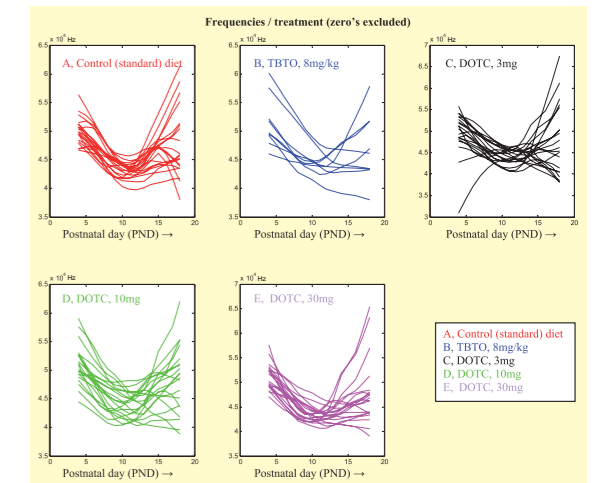


Figure 4. Ultrasonic vocalizations (USV) in rat pups (PND 4 to 18). Effects of organotin compounds DOTC (3 dose groups) and TBTO (in daily diet) on the frequency of USV calls per test day. Compare groups A and B and notice that the 'dip' of frequency in the control diet group A (at about PND 11) is shifted slightly to a later postnatal day in the TBTO group B. See also figure 3.