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Effects of intracerebroventricular administration of dimethyl sulfoxide on hippocampal electrophysiology in mice

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ABSTRACT

Dimethyl sulfoxide (DMSO) is a commonly used solvent in life sciences due to its excellent ability to dissolve compounds with poor water-solubility. Depending on the applied dose, the variety of DMSO's physiological and biological effects may compromise its suitability as a vehicle molecule. Even low concentrations of DMSO are known to affect neuronal excitability *in vitro*. As *in vivo* effects have not been studied extensively, this exploratory study investigated the effects of intracerebroventricular (ICV) administration of different DMSO concentrations on hippocampal electrophysiology in mice. Acute recordings of hippocampal evoked potentials (EPs) and electroencephalography (EEG) were performed before and after ICV injection of a 5 µl DMSO solution, with concentrations ranging from 2.5 % to 100 % DMSO. Solutions containing up to 50 % DMSO had no acute effects on hippocampal electrophysiology. Administration of 75 % and 100 % DMSO was found to alter evoked responses, indicating increased excitability. Our results indicate that DMSO can be used as a vehicle in volumes of 5 µl containing concentrations of up to 50 % without affecting acute hippocampal electrophysiological studies in mice. Higher concentrations should be avoided as these affect neuronal excitability.

1. Introduction

Dimethyl sulfoxide (DMSO) is a small, organic, amphiphilic molecule consisting of a polar sulfoxide group and two apolar methyl groups (Fig. 1). Its chemical properties allow for very efficient dissolving of hydrophobic compounds and make DMSO one of the most widely used solvents in biomedical research (Santos et al., 2003). DMSO is also commonly used as a cryoprotectant for cell transplantations (Awan et al., 2020) and has several clinical applications based on the discovery of immunomodulatory, neuroprotective and cardiovascular effects possessed by this molecule (Huang et al., 2020; Jacob and de la Torre, 2009; Santos et al., 2003). Due to this variety in physiological and biological activity, the amount of DMSO that can be used as a vehicle for water-insoluble drugs in experimental studies is often limited when confounding effects are undesirable.

In neuroscience research, DMSO is frequently used as a vehicle both for *in vitro* and *in vivo* experimental studies. Multiple *in vitro* studies have reported effects of DMSO on various electrophysiological read-out parameters such as a depression of Na^+, K^+ and Cl^- currents (Ogura et al., 1995), suppression of glutamate-induced AMPA- and NMDA- mediated ion currents (Lu and Mattson, 2001) and changes in intrinsic excitability (Tamagnini et al., 2014). While intracerebroventricular (ICV) injections of DMSO-containing solutions are commonly used for initial testing of biological activity of less soluble compounds in the brain, the *in vivo* effects of DMSO have been studied less extensively. A wide range of DMSO dosages with concentrations between 5 % and 100 % in volumes ranging from 1 to 5 µl have been used to deliver compounds to the CNS (Benedykcinska et al., 2016; Echeverry et al., 2007; Kim et al., 2016; Ohinata et al., 2008; Shen et al., 2021; Vahidy et al., 2006; Xu et al., 2010). Given the demonstrated *in vitro* effects of DMSO on neuronal excitability, an effect of ICV administration of DMSO in *in vivo* experiments can be expected. Only one study has previously investigated this by studying acute effects on electrophysiological characteristics of neurons in the rat barrel cortex (Soltani et al., 2016). No changes were found in neuronal spontaneous activity and evoked responses to stimulation of whiskers upon ICV administration of 5 µl of 10 % DMSO.

This study now explored the effects of different DMSO concentrations dissolved in saline (2.5–100 % v/v in a total volume of 5 µl) on hippocampal spontaneous EEG activity and evoked potentials (EPs) recordings. This latter electrophysiological technique records the neuronal

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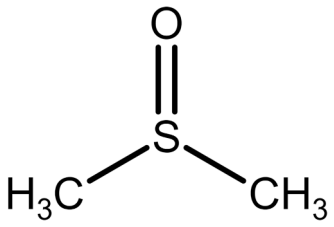

Fig. 1. Chemical structure of dimethyl sulfoxide.

responses of granule cells at the dentate gyrus (DG) of the hippocampus evoked by electrical stimulation of the perforant path (PP) and is used to investigate changes in neurotransmission and neuronal excitability. Stimulation of presynaptic axons of the PP causes depolarization of postsynaptic granule cells in the DG, which generates a field excitatory postsynaptic potential (fEPSP). Synchronous generation of action potentials in the granule cells induces a "population spike (PS)", visible as a negative deflection on the fEPSP. We investigated changes in these parameters and performed a spectral analysis of hippocampal EEG to investigate which range of DMSO concentrations can be used as vehicle solution in studies exploring acute effects of ICV administered compounds on hippocampal electrophysiology.

2. Materials & methods

2.1. Animals

Adult male C57Bl/6J mice (2–3 months of age, n = 21) were obtained from Envigo (The Netherlands). The animals were group housed under environmentally controlled conditions (temperature 21–22°C, relative humidity 40–60 %) at a fixed 12-h light/dark cycle with food and water available *ad libitum*. Experiments were conducted in accordance to the European Directive 2010/63/EU and were approved by the Animal Experimental Ethical Committee of Ghent University (ECD 20/21).

2.2. Stereotaxic surgery

Mice were anesthetized with isoflurane (5 % at 2 l/min for induction, 2 % at 0.5 l/min for maintenance) and fixed in a stereotaxic frame (Stoelting, USA). A constant body temperature was maintained using a rectal probe connected to a heating pad. After exposing the skull, holes were drilled for intracerebroventricular (ICV) injection and placement of electrodes. An epidural screw electrode (stainless steel, 1.57 mm diameter, Bilaney Consultants, Germany) was placed in the frontal bone and used as the ground/reference electrode. Bipolar depth electrodes, consisting of two twisted polyimide-coated stainless-steel wires of 70 μm (recording electrode) and 120 μm (stimulation electrode) diameter (California Fine Wire, USA), were stereotactically implanted. The bipolar recording electrode (200 μm tip separation) was implanted in the dentate gyrus (DG) of the left hippocampus (coordinates: −2.0 mm AP and +1.3 mm ML relative to bregma, −2.0 mm DV relative to dura). The bipolar stimulation electrode (500 μm tip separation) was implanted in the perforant path (coordinates: −3.7 mm AP and +2.0 mm ML relative to bregma, −1.5 mm DV relative to dura). The dorsoventral position of the depth electrodes was adjusted using electrophysiological feedback until optimal evoked potential (EP) waveforms were obtained. Next, a 5 μl Hamilton neuros syringe (Hamilton Company, USA) was inserted in the right lateral ventricle (coordinates: −0.3 mm AP and −1.0 mm ML relative to bregma, −2.2 mm DV relative to dura), after which recordings were initiated.

2.3. Recording procedure

Hippocampal spontaneous activity and EPs were recorded using a custom MATLAB-based script (MathWorks, USA), controlling a USB-6211 NI-DAQ card (National Instruments, USA) for data acquisition and stimulation. Recordings were high-pass filtered at 0.1 Hz, amplified 248 times, digitized at 10 kHz (16-bit resolution, input range of ± 10 V) and stored on a PC for off-line analysis. Every 10 s, a 6-s sweep of electroencephalography (EEG) was recorded. At the start of every sweep, the perforant path was stimulated by delivering biphasic square-wave pulses (200 μs per phase) through the stimulation electrode, generated by a constant current linear stimulus isolator (Digitimer, UK). First, input-output (I/O) curves were constructed by stimulating at increasing current intensities (50–500 μA in increments of 50 μA, 500–1000 μA in increments of 100 μA), repeated four times. The stimulation intensity evoking ± 50 % of the maximal population spike amplitude on the averaged I/O curve was then determined and used for the subsequent EP recordings.

Baseline EP recordings were conducted for at least 20 min. Then, 5 μl of DMSO solution was injected at a rate of 10 μl/min with the Hamilton syringe controlled by a Quintessential Stereotaxic Injector (Stoelting, USA). DMSO (Tocris Bioscience, Bristol, UK) was diluted in physiological saline solution (0.9 % NaCl). A range of 7 different DMSO concentrations (% v/v) were tested: 2.5 % (n = 4), 12.5 % (n = 3), 25 % (n = 4), 37.5 % (n = 3), 50 % (n = 4), 75 % (n = 5) and 100 % (n = 5). Immediately after the injection, recordings were continued for 60 min. After conclusion of the recordings, animals were euthanized with an intraperitoneal injection of sodium pentobarbital (1500 mg/kg).

2.4. Data analysis and statistics

Evoked potential recordings were analyzed using a custom script in MATLAB (MathWorks, USA) software, measuring the slope of the field excitatory post-synaptic potential (fEPSP) and the amplitude of the population spike (PS). The fEPSP slope was calculated by fitting a tangent to the initial rising phase of the fEPSP. The PS amplitude was calculated as the distance between the negative peak of the PS and the line connecting the positive peaks before and after the PS. Values were averaged per 5 min (i.e. for 30 EPs) and normalized to the mean of the 20-min baseline period.

Power spectra were constructed from the hippocampal EEG obtained from the last 5 s of each 6-s sweep, averaged per 5 min. Spectral analysis was performed using custom scripts in Python (v3.8.5, Python Software Foundation). The differential EEG signals derived from the bipolar electrode in the hippocampus were high-pass filtered at 1 Hz (1st-order Butterworth) and segmented into 1-s epochs with 50 % overlap. These segments were windowed (Blackmann-Harris) after which the Fast Fourier algorithm was used to compute the power spectra between 1 and 100 Hz. Frequencies around 50 Hz (48–52 Hz) were excluded to avoid power line interference. Power values were log-transformed into decibels (10 *log10), the sum of the total power (1–100 Hz) was taken and the difference in power to baseline was calculated.

Differences between the effects of the different DMSO concentrations were statistically analyzed using a restricted maximum likelihood (REML) linear mixed effects model. The fEPSP amplitude, the PS amplitude or EEG power was selected as dependent variable. DMSO concentration (group), time relative to injection (time) and group by time interaction were used as fixed factors and animal ID as random factor. The first-order autoregressive model was used as covariance structure to account for repeated measures over time. Bonferroni correction was used for *post-hoc* comparison of timepoints between groups. A p-value < 0.05 was set for statistical significance. Group values are reported and plotted as means ± standard error of the mean.

3. Results

The effects on hippocampal evoked potentials were measured after ICV injection of 5 μl of 7 different DMSO concentrations, ranging from 2.5 % to 100 %. Administration of 2.5 % (n = 4), 12.5 % (n = 3), 25 %

(n = 4), 37.5 % (n = 3) and 50 % (n = 4) DMSO did not significantly affect the fEPSP slope or PS amplitude. Injections of 75 % DMSO (n = 5) and 100 % (n = 5) DMSO led to clear changes shortly after injection in the majority of animals. Administration of the 75 % DMSO solution resulted in a major increase in PS amplitude in 3 out of 5 animals. of which one also displayed a clear increase in fEPSP slope (Supplementary Fig. S1 A). Upon injection of 100 % DMSO, the PS amplitude greatly increased in 4 out of 5 animals (Supplementary Fig. S1 B). This was paired with an increase in fEPSP slope in 2 animals. On average, the PS amplitude in the 75 % and 100 % DMSO groups reached maximum values of 174 ± 35 % and 279 ± 75 % of the baseline value respectively, 15–20 min after injection (Fig. 2). In the 100 % DMSO group, the fEPSP slope increased to a maximum of 167 ± 65 % of the baseline value 10–15 min after injection. There was no increase of the slope with 75 % DMSO at group level. Statistical analysis confirmed a difference between groups for the PS amplitude ($F = 2.544$, $p = 0.021$). No significant difference could be found for the fEPSP slope ($F = 1.134$, $p = 0.367$). Post-hoc comparison of the differences in PS amplitude between groups revealed that the amplitude in the 100 % DMSO group was significantly different compared to the groups with 2.5–50 % DMSO at 10–35 min after injection (see Supplementary Table S1).

Spectral analysis of the hippocampal EEG indicated some effects on spontaneous activity after ICV administration of high concentrations of DMSO. However, at group level, only the 100 % DMSO group displayed a noticeable decrease in EEG power over time compared to baseline (Fig. 3). Ten to twenty minutes after injection of 100 % DMSO solution, when effects on the EPs were maximal, the total EEG power (1–100 Hz) was reduced with 331 ± 166 dB. The differences between groups did not reach statistical significance in our linear mixed model analysis ($F = 0.962$, $p = 0.471$).

4. Discussion

We investigated the effects of ICV administration of different DMSO doses on hippocampal EPs and spontaneous EEG activity in mice. With an injection of 5 µl, solutions containing DMSO concentrations of up to 50 % did not significantly affect EP parameters, nor did it affect EEG power. At concentrations of 75 % and 100 %, DMSO caused acute increases in the fEPSP slope and PS amplitude in part of the animals. The population spike was most sensitive to changes caused by DMSO administration, resulting in a significant effect on PS amplitude in the 100 % DMSO group. No significant effects on hippocampal EEG were found, but our data suggest a decrease in EEG power in the 1–100 Hz spectrum with a concentration of 100 %.

The increases in neuronal evoked responses after administration of DMSO *in vivo*, as we demonstrated here using hippocampal EP recordings, have not been described before. Previous *in vitro* studies have primarily reported suppressive effects on neuronal excitability upon exposure to DMSO. In primary hippocampal cell cultures, a 5-min exposure to 5 % DMSO was shown to reduce glutamate-induced excitatory currents and intracellular Ca^{2+} responses (Lu and Mattson, 2001). In another study on murine brain slices, pre-treatment with a much lower concentration of 0.05 % DMSO reduced intrinsic excitability of hippocampal pyramidal cells when measured with subthreshold stimuli (Tamagnini et al., 2014). In this study more complex neurophysiological changes were also found when investigating action potential generation using larger depolarizing currents. On the one hand, DMSO treated cells displayed reductions in both the number of spikes fired and their firing frequency, indicating again a decrease in excitability. On the other hand, a nearly significant increase in the action potential peak amplitude and a significant negative shift in action potential threshold were observed which, as the authors noted, could facilitate action potential generation under some circumstances. This could be an explanation for the increases in PS amplitude observed in our study *in vivo*. The discrepancies between the increased neuronal responses observed in our *in vivo* results and the previously reported decreases in excitability from *in vitro* studies might be partly due to the differences in experimental settings. The neuronal responses generated by a population of cells recorded in the

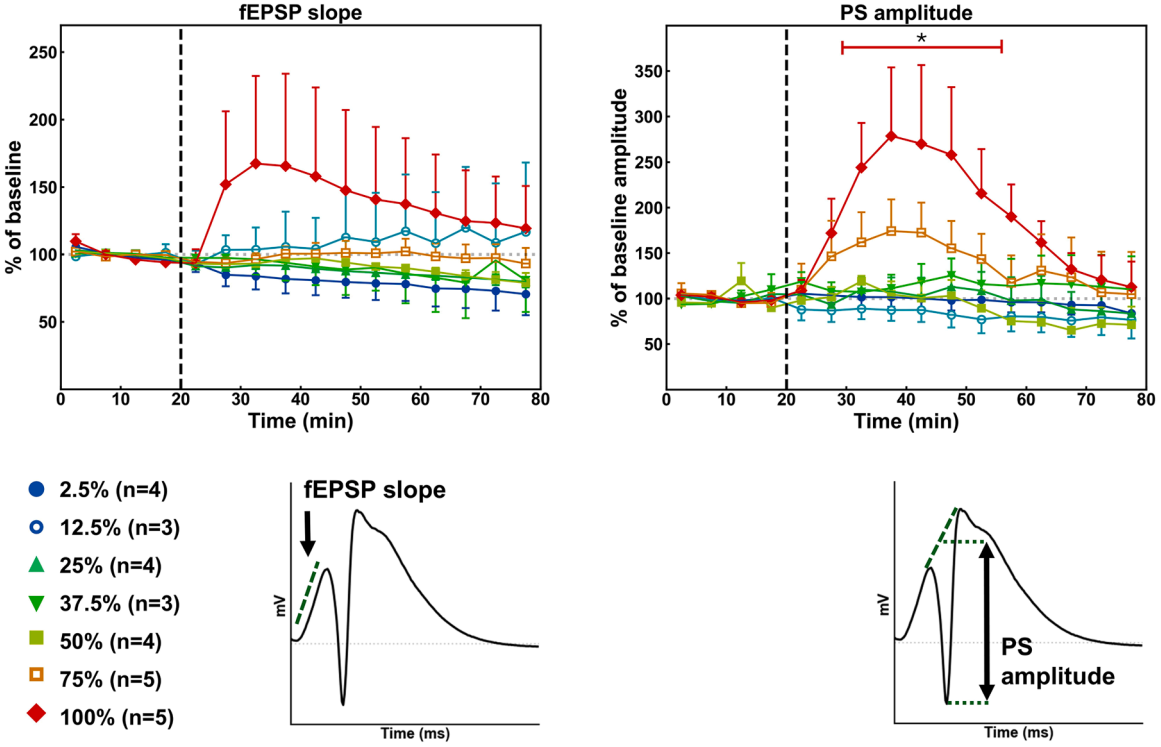

Fig. 2. Effects of ICV injection of different DMSO concentrations on hippocampal evoked potentials. Changes in field excitatory post-synaptic potential (fEPSP) slope and population spike (PS) amplitude, normalized to baseline, plotted over time. Dashed vertical line indicates time of injection. * indicates timepoints where the 100 % DMSO group (red) significantly differs from other groups with $p < 0.05$.

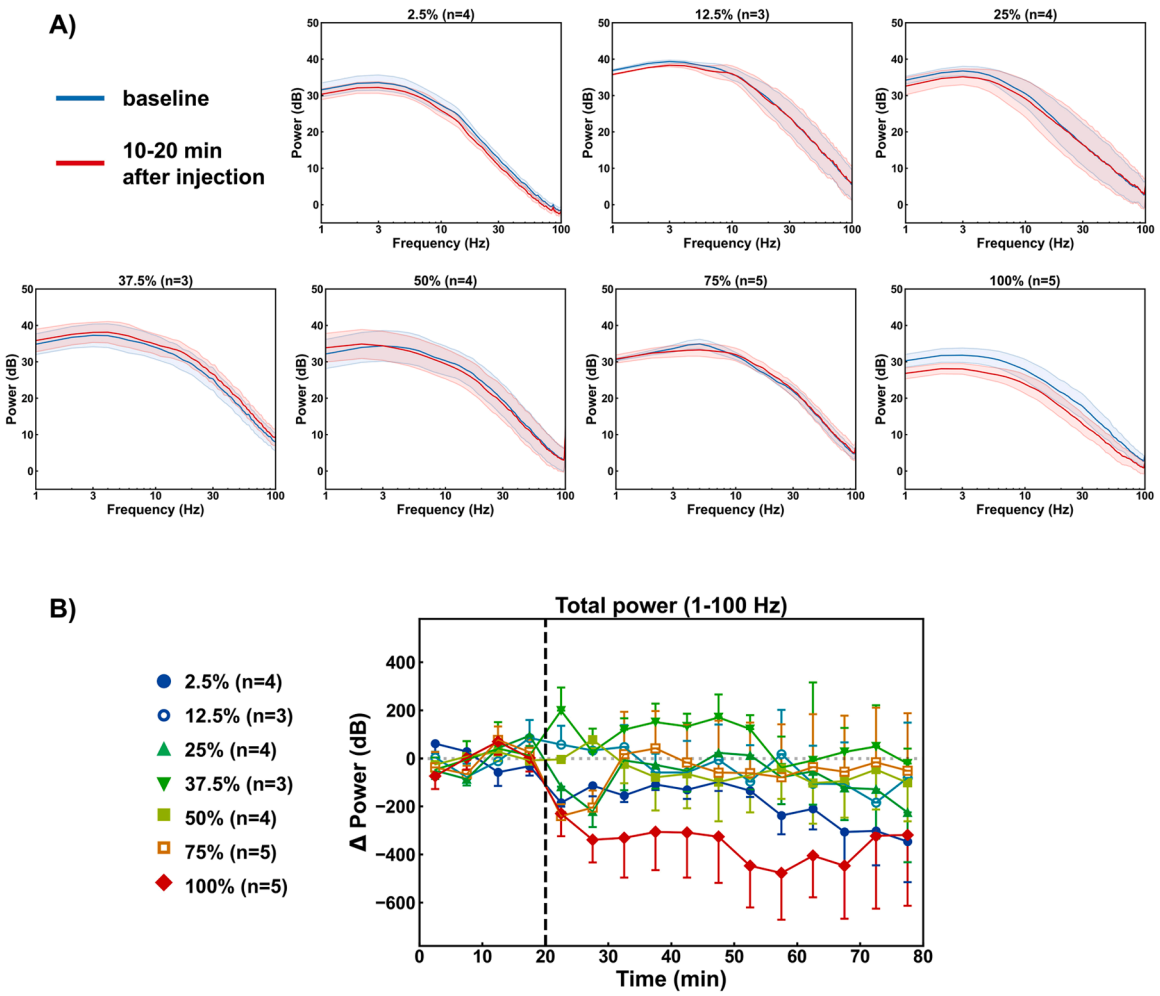

Fig. 3. Effects of ICV injection of different DMSO concentrations on hippocampal electroencephalography (EEG). (A) EEG power spectra before (minute 0–20, blue) and 10 min after (minute 30–40, red) ICV injection of the different DMSO concentrations. (B) Change over time of the total EEG power (1–100 Hz) compared to baseline. Dashed vertical line indicates time of injection.

intact *in vivo* brain are much more complex compared to the single cell recordings *in vitro*. The *in vivo* hippocampal EPs are modulated by the interplay of both excitatory and inhibitory networks. DMSO has been shown to block GABA-induced Cl^- currents at concentrations of 0.3–3 % *in vitro* (Nakahiro et al., 1992), so this could also play a part in the observed increase in hippocampal excitability after ICV administration of DMSO. Additionally, the use of general anesthesia could also influence the observed effects of DMSO in this *in vivo* study, as isoflurane is known to modulate the excitability of hippocampal neurons through effects on Na^+ currents and the membrane potential (Berg-Johnsen and Langmoen, 1990; Ou et al., 2020; Zhao et al., 2021). Besides its effects on neurons, DMSO administration is known to affect astrocytes as well, which could also be involved in the changes in excitability observed in the present study. Astrocytes modulate neuronal excitability via homeostatic regulation of the extracellular environment, such as glutamate and ion concentrations (Verhoog et al., 2020). Especially through the regulation of extracellular potassium concentrations, known as spatial K^+ buffering, astrocytes play a vital role in balancing neuronal excitability, and impaired K^+ buffering leads to hyperexcitability (Bellot-Saez et al., 2017; Wallraff et al., 2006; Xu et al., 2009). Since exposure of astrocyte cultures to ≥ 5 % DMSO has been shown to lead to reduced cell viability (Castaneyra-Ruiz et al., 2024; Yuan et al., 2014; Zhang et al., 2017), a disruption of the astrocytic K^+ homeostasis could be another reason why DMSO administration causes an increase in neuronal excitability.

While DMSO affects electrophysiology *in vitro* at concentrations even below 1 %, clear effects were only present in our *in vivo* experiment after ICV injection of 75 % and 100 % DMSO solutions. Of course, the actual *in situ* concentration that hippocampal tissue will be exposed to *in vivo* after an ICV injection will be much lower due to dilution of the injected solution in the cerebrospinal fluid (CSF). The total CSF volume in adult mice is about 35–40 µl (Lim et al., 2018; Rudick et al., 1982), meaning that a 5 µl solution would be diluted 8–9 times assuming complete diffusion in the CSF. For a 50 % DMSO concentration, which is the highest concentration tested without clear effects, this would result in a concentration of $\pm$ 6 % in the CSF. Additionally, the actual *in situ* concentration would still be reduced by a factor depending on the vicinity to the ventricular system. Even though the hippocampus borders the lateral ventricle, our results now suggest that ICV injections of up to 50 % DMSO in 5 µl could still be used safely as a vehicle without causing confounding effects on electrophysiology. So, if needed for the ICV administration of poorly soluble compounds, a DMSO concentration above 10 % could be used, which was previously reported to be a safe concentration for electrophysiological studies (Soltani et al., 2016).

From our study it is clear that high amounts of DMSO can strongly alter excitability and should preferably be avoided. While not frequently used, some studies have used ICV injections of 5 µl of 100 % DMSO (Benedykcinska et al., 2016; Vahidy et al., 2006). In such cases, it is thus of utmost importance to include adequate vehicle control groups. We have now shown that this concentration affects neuronal functioning.

Injection of a 75 % DMSO solution also shows potential effects on excitability, but due to the limited sample sizes in this exploratory study only the 100 % DMSO condition yielded significant effects in our linear mixed effects model. However, our current results could be used to design future studies with adequate power to investigate the limits of ICV injections of DMSO in more detail. It is also not yet fully conclusive from our study how the EEG is affected, but there seems to be an indication of a longer lasting, gradual decrease in EEG power, which will require further research. It is also important to note that currently only the acute effects of a single ICV injection have been investigated. Certain DMSO concentrations used in acute experiments could be less suitable for repeated injections in chronic studies. So dependent on the type of study and the outcome parameters under investigation, the use of high amounts of DMSO still has to be carefully considered. Another limitation of the current study is that effects were only investigated in male mice. As sex differences are known to exist in certain aspects of neuronal excitability and synaptic transmission (Jain et al., 2019; Proaño et al., 2018; Tabatadze et al., 2015), potential sex-specific effects of DMSO should be taken into account before generalizing the current results.

To summarize, we tested the effects of increasing DMSO concentrations on hippocampal electrophysiology and observed increases in evoked potentials after ICV administration of 5 µl of 75 % and 100 % DMSO. Solutions up to 50 % of DMSO did not cause electrophysiological effects, indicating that these concentrations could be suitable as vehicle for acute ICV administration of compounds with poor water solubility.

Compliance with ethical standards

Experiments were conducted in accordance to the European Directive 2010/63/EU and were approved by the Animal Experimental Ethical Committee of Ghent University (ECD 20/21).

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CRediT authorship contribution statement

Kristl Vonck: Writing – review & editing, Supervision, Funding acquisition. **Paul Boon:** Supervision, Project administration, Funding acquisition. **Robrecht Raedt:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Evelien Carrette:** Writing – review & editing, Project administration. **Jeroen Spanoghe:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Arne Van Acker:** Investigation, Formal analysis, Conceptualization.

Declaration of Competing Interest

None.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ibneur.2025.02.016.

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