Accepted Manuscript

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PII: S0278-5846(11)00377-0
DOI: doi: [10.1016/j.pnpbp.2011.12.012](https://doi.org/10.1016/j.pnpbp.2011.12.012)
Reference: PNP 8080

To appear in: *Progress in Neuropsychopharmacology & Biological Psychiatry*

Received date: 1 November 2011
Revised date: 19 December 2011
Accepted date: 25 December 2011

Please cite this article as: O'Donovan Sinead, Kennedy Mark, Guinan Blaithin, O'Mara Shane, McLoughlin Declan M., A comparison of brief pulse and ultrabrief pulse electroconvulsive stimulation on rodent brain and behaviour, *Progress in Neuropsychopharmacology & Biological Psychiatry* (2011), doi: [10.1016/j.pnpbp.2011.12.012](https://doi.org/10.1016/j.pnpbp.2011.12.012)

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A comparison of brief pulse and ultrabrief pulse electroconvulsive stimulation on rodent brain and behaviour

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Abstract

Brief pulse electroconvulsive therapy (BP ECT; pulse width 0.5-1.5 msec) is a very effective treatment for severe depression but is associated with cognitive side-effects. It has been proposed that ultrabrief pulse (UBP; pulse width 0.25-0.30 msec) ECT may be as effective as BP ECT but have less cognitive effects because it is a more physiological form of neuronal stimulation. To investigate this further, we treated normal rats with a 10 session course of either BP (0.5 msec), UBP (0.3 msec), or sham electroconvulsive stimulation (ECS) and measured antidepressant-related changes in dentate gyrus cell proliferation and hippocampal BDNF protein levels as well as hippocampal-dependant spatial reference memory using the water plus maze and immobility time on the forced swim test. Both BP and UBP ECS induced very similar types of motor seizures. However, BP ECS but not UBP ECS treatment led to a significant, near 3-fold, increase in cell proliferation ($p=0.026$) and BDNF levels ($p=0.01$). In the forced swim test, only BP ECS treated animals had a significantly lower immobility time ($p=0.046$). There was a trend for similarly reduced hippocampal-dependent memory function in both BP and UBP groups but overall there was not a significant difference between treatment and control animals when tested 10 days after completing allocated treatment. These findings show that, even though both forms of ECS elicited similar motor seizures, UBP ECS was less efficient than BP ECS in inducing antidepressant-related molecular, cellular and behavioural changes.

Key words: pulse width, electroconvulsive therapy, electroconvulsive stimulation, depression, BDNF

Abbreviations: ECT, electroconvulsive therapy; ECS, electroconvulsive stimulation; BP, brief pulse; UBP, ultrabrief pulse; BDNF, brain derived neurotrophic factor; FST, forced swim test.

1. Introduction

Electroconvulsive therapy (ECT) is the most acutely effective treatment available for severe, often treatment resistant, depression with remission rates of about 60% (Eranti et al., 2007; Group, 2003; Kellner et al., 2010). However, its use is limited by concerns about adverse effects on memory and executive function arising during treatment though these mostly resolve within weeks of finishing a course of ECT (Semkovska et al., 2011; Semkovska and McLoughlin, 2010). Since its introduction over 70 years ago, there have been several modifications to ECT technique to reduce such cognitive side-effects while maintaining its effectiveness. These have focussed mainly on electrode placement and electrical stimulus parameters. While there seems to be little difference between bitemporal and bifrontal electrode placement (Dunne and McLoughlin, 2011), right unilateral electrode placement is associated with less adverse cognitive effects than bitemporal ECT but has less of an anti-depressant effect (Sackeim et al., 1993). To improve effectiveness of unilateral ECT higher stimulus doses above seizure threshold are required but this is associated with increasing dose-related cognitive deficits (McCall et al., 2000; Semkovska et al., 2011). Recent studies have confirmed that high-dose unilateral ECT is as effective as modestly suprathreshold bitemporal ECT but it is not yet clear that it has substantially less cognitive adverse effects (Kellner et al., 2010).

Changes in electrical stimulus parameters have improved the efficiency of neuronal stimulation. The move from a sine-wave stimulus to brief pulse (i.e. 0.5 – 1.5 msec) square wave stimulus, requiring less energy, has reduced cognitive deficits but not compromised clinical effectiveness (UK ECT Review Group, 2003; Semkovska and McLoughlin, 2010). More recently there has been interest in using ultrabrief (i.e. 0.25 – 0.3 milliseconds) pulse stimulation as this is more physiological, reducing stimulation of neurones that are already depolarising or in the refractory period (Sackeim, 2004). The first randomised trial reported a high remission rate (77%) and few cognitive deficits following high-dose right unilateral ultrabrief pulse ECT compared to brief pulse ECT (Sackeim et al., 2008). However, this high remission rate has so far not been replicated in other randomised (6-44%; (Quante et al., 2011; Sienaert et al., 2009)) and non-randomised studies (13-42%; (Loo et al., 2007; Loo et al., 2008; Niemantsverdriet et al., 2011)). Like brief-pulse unilateral ECT, one possibility is

that a higher stimulus charge is required and the optimal stimulus parameters remain to be established.

Electroconvulsive stimulation (ECS) is the animal model equivalent of ECT. Much has been reported on molecular and cellular changes induced by courses of repeated brief-pulse ECS in both normal rats and rat models for depression and that are believed to be relevant to antidepressant mechanisms, e.g. up-regulation of neurotrophic factors and hippocampal cell proliferation and neurogenesis (Gersner et al., 2010; Madsen et al., 2000; Newton et al., 2003). In contrast, little has been reported on such effects following ultrabrief-pulse ECS. Here we aimed to compare the effects of brief pulse and ultra-brief pulse ECS on normal rodent brain and behaviour. We sought to determine if both forms of brain stimulation would induce similar antidepressant-related changes in hippocampal cellular proliferation and brain derived neurotrophic factor (BDNF) expression. Additionally, as secondary measures, we sought to identify if there were any differences in antidepressant-related behavioural changes and cognitive function that could be attributed to the difference in stimulus pulse width.

2. Experimental procedures

2.1. Animals and treatment

Male Sprague-Dawley rats (Harlan, UK), weighing 150-200g at intake, were housed in groups of four with food and water available *ad libitum* under 12 hour light-dark conditions. Animals were allowed to habituate for ten days prior to ECS. Experiments were conducted in accordance with EU directive 2010/63/EU and guidelines of the Bioresources Ethics Committee, Trinity College Dublin.

The experimental design is summarised in Figure 1A. Animals were randomly allocated to a course of bilateral brief pulse (BP), ultrabrief pulse (UBP), or sham ECS. ECS was delivered thrice weekly (Monday, Wednesday, Friday) for ten sessions via ear-clip electrodes using the ECT Unit 57800 device (Ugo Basile, Italy). Parameters for BP ECS were: 0.5ms pulse-width; 100 pulses/second; 0.7s duration; 75mA current; while for UBP ECS the pulse-width was reduced to 0.3ms. Sham animals were identically handled but received no charge. Tonic and clonic seizure durations were recorded on the first, fifth and tenth ECS

sessions. The tonic phase of the seizure was defined as the period starting at the end of stimulus administration during which an animal's hind legs and forearms were held rigidly against the body with some muscle twitching until the beginning of the clonic phase during which the forearms and legs extended stiffly away from the body and began flexing - extending rapidly until such motor activity was no longer apparent.

2.2. Behavioural studies

2.2.1. Water plus maze

Animals were habituated to the behavioural testing area for one week after completing the allocated course of ECS. Hippocampal-dependent spatial reference memory was assessed using a water plus maze (Diamond et al., 1999). The maze had four closed arms in a plus formation with a central rectangular region and was filled with water ($21\pm1^{\circ}\text{C}$) to a depth of 45cm. A hidden platform, the same colour as the maze, was placed 2.5cm below the level of the water and located at the distal end of one arm. Visual cues were positioned on surrounding walls. Each animal was placed, facing away from the centre, into the distal end of a pre-selected entry arm on either the left or right of the hidden platform arm for all trials on days 1-3 of testing and completed 10 such trials daily (60s/trial; 30s inter-trial intervals). On day 4, animals underwent 30 trials in which platform position did not change but entry into arms was pseudo-randomised so that all animals started 10 trials in each of the other three different arms. Frequency of platform acquisition, as a measure of reference memory, was calculated. Velocity (cm/s) for each treatment group and platform acquisition on each experiment day was also measured. EthovisionXT (Noldus Information Technology, The Netherlands) was used for data collection.

2.2.2. Forced swim test

The forced swim test, a measure of responsiveness to antidepressant treatments (Porsolt et al., 1977), was conducted in two sessions on days 12-13 after completing the course of ECS, consisting of a 15 minutes pre-test followed by a 5 minutes test 24 hours later. A 50cm high, 25cm diameter, clear cylinder was filled 35cm from the base with water ($23\pm1^{\circ}\text{C}$). The length of time animals spent immobile was measured over the 5 minutes test session. Immobility behaviour was recorded when animals were making only movements

required to stay afloat. Animals were sacrificed 24 hours after the swim test and the hippocampus was immediately dissected, snap-frozen in liquid nitrogen and stored at -80°C .

2.3. BDNF expression

Hippocampal BDNF protein levels were measured using a sandwich ELISA kit according to the manufacturer's instructions (Chemikine, Chemicon (Millipore), USA). Briefly, hippocampal samples were homogenised in the recommended lysis buffer (10% w/v). Homogenates were centrifuged at 14,000g for thirty minutes and the supernatant was collected for use in the BDNF assay. The amount of BDNF in each sample was analysed in duplicate and determined from the regression line generated by BDNF standards (range 7.8pg/ml to 500pg/ml). Total protein concentrations were measured by Bradford protein assay. BDNF results are presented as pg BDNF per mg of total protein content in each sample.

2.4. Immunohistochemistry

To label proliferating cells in the dentate gyrus, a second set of animals was similarly treated with ECS and administered BrdU (40 mg/kg, i.p.; Sigma-Aldrich) 2-4 hours post ECS on treatment days three to ten (see Figure 1B). Twenty-four hours after the final ECS, animals were anaesthetised with urethane and transcardially perfused with 0.9% saline, followed by 4% paraformaldehyde, essentially as previously described (Kesavapany et al., 2002).

Hippocampal coronal sections (10 μm) were mounted on slides for staining. Unless specified otherwise, all procedures took place at room temperature. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide solution. Slides were washed for 10 minutes under running water, microwaved on full power for 13 minutes in 10mM citric acid buffer (pH6.0), washed in TBS, incubated in avidin (Vector Laboratories) for 10 minutes, washed twice in TBS and then incubated in biotin (Vector Laboratories) for 10 minutes. Following two washes in TBS and incubation in 10% normal rabbit serum (Dako) for 5 minutes, sections were incubated with rat anti-BrdU (1:10,000; ab6326, Abcam) in TBS for 1 hour. After this, slides were washed thrice in TBS and incubated in biotinylated rabbit anti-rat secondary antibody (1:50; Vector Laboratories) in TBS for 30 minutes. Following two

TBS washes, StrepAB/HRP complex (Vector Laboratories) was incubated on each slide for 30 minutes. Slides were washed once in TBS and twice in distilled water before a 15 minute DAB (SigmaFAST DAB tablets) incubation to visualise the reaction product. Following a wash with distilled water, sections were counterstained with Hematoxylin. Sections were imaged using an Olympus DP72 at 20x magnification. Numbers of BrdU-positive cells in dentate gyrus subgranular zone and granule cell layer were counted blind on coded slides from every sixth section over a range of 24 sections per animal.

2.5. Statistical analyses

Data are presented as means $\pm$ SEM and were analysed using GraphPad Prism version 5.0 (Graphpad Software, La Jolla California, USA). A repeated measures ANOVA was used to compare seizure durations between groups. One-way ANOVAs were used to compare ELISA data and probe day platform acquisition for the water plus maze with Newman Keuls post-hoc tests as required. Repeated measures ANOVA, with Day (water plus maze Training days 1, 2, 3) or Treatment (BP ECS, UBP ECS, sham ECS) as factors, was used to analyse platform acquisition during training on the water plus maze with Bonferroni post tests as required. Kruskal-Wallis test with Dunn's multiple comparison post hoc tests was used to analyse BrdU-positive cell counts. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Tonic-clonic seizure durations

Tonic and clonic seizure durations were compared on ECS treatment days 1, 5 and 10 (Figure 2). Repeated measures ANOVA showed that both tonic ($F_{(2,20)}=3.88$, $p=0.038$) and clonic ($F_{(2,20)}=18.63$, $p<0.0001$) seizure durations lengthened with increasing number of treatments. Tonic seizure durations have previously been similarly reported to increase over time in a study comparing the effects of ECS using pulse widths of either 0.5 ms or 10 ms (Jansson et al., 2009). However, durations of the tonic ($F_{(1,20)}=0.27$, $p=0.616$) or clonic ($F_{(1,20)}=0.82$, $p=0.386$) seizure phases did not significantly differ between BP and UBP groups.

3.2. Behavioural changes after ECS

Water plus maze platform acquisition on training and probe days were investigated to determine if animals learned the platform position and could recall this information. Platform acquisition during the training period increased significantly over time ($F_{(2,28)}=7.23$, $p=0.0029$) but was not affected by treatment type ($F_{(2,28)}=0.41$, $p=0.67$; Figure 3A), indicating that all animals learned the task, and there was no group x time interaction effect ($F_{(4,28)}=0.48$, $p=0.7513$). On the probe trial day, both BP and UBP ECS-treated animals obtained the platform less frequently than controls but this difference was not statistically significant ($F_{(2,14)}=1.8$, $p=0.21$; Figure 3B). Of note, there was no difference between the levels of platform acquisition by BP and UBP groups. The swimming velocity (cm/s) at which the animals obtained the platform increased significantly ($F_{(2,42)}=6.47$, $p=0.004$) over the three training days but did not differ between the three treatment groups ($F_{(2,42)}=0.16$, $p=0.849$), indicating no motor deficit resulted as a consequence of real ECS treatments. Similarly, no significant difference in velocity to finding the platform was found between the three groups ($F_{(2,9)}=0.033$, $p=0.967$) on the probe day.

The groups differed with regards to immobility times in the Forced Swim Test ($F_{(2,15)}=3.8$, $p=0.046$; Figure 4). Post hoc analysis revealed that the BP group showed a significant decrease ($p<0.05$) in immobility time when compared to controls, whereas a non-significant decrease in immobility time was seen in the UBP group.

3.3. BDNF protein levels after ECS

Different levels of hippocampal BDNF were found between the three groups ($F_{(2,15)}=6.3$, $p=0.01$) when measured two weeks after completing the allocated course of ECS with BP ECS producing significantly higher levels than found in both the control ($p<0.01$) and UBP ($p<0.05$) groups (Figure 5). A smaller, but non-significant, increase was found in the UBP group compared to sham-treated controls.

3.4. Immunohistochemistry

ECS treatment was associated with an increase in cell proliferation compared to sham treatment ($p=0.026$, Figure 6) when measured 24 hours after completion of the

allocated course of ECS. BP ECS induced a significant ($p < 0.05$), almost 3-fold, increase in the number of proliferating cells compared to sham treated control animals, whereas a much smaller and non-significant increase in cell proliferation was seen following UBP-ECS.

4. Discussion

To our knowledge, this is the first study to systematically compare the effects of UBP (0.3 ms) and BP (0.5 ms) width stimuli on chronic ECS and related antidepressant-related changes in rats. We found that both forms of ECS elicited seizures that were indistinguishable with regards to observable motor seizure activity and the durations of the tonic and clonic phases. However, while BP ECS induced significant increases in recognised markers of antidepressant action, UBP ECS did not have the same effect.

Compared to sham-treated animals, a large, three-fold, and significant increase in cell proliferation was seen within the dentate gyrus one day after completing a course of BP ECS while only a much smaller, non-significant, increase was seen following UBP ECS. This degree of increased cell proliferation following BP ECS is similar to that previously found by other groups (Madsen et al., 2000; Segi-Nishida et al., 2008). The majority (75-88%) of these new cells have been reported to be neuronal (Malberg et al., 2000; Scott et al., 2000) and such hippocampal neurogenesis is required for anti-depressant behavioural effects in both rats and nonhuman primates (Perera et al., 2011; Santarelli et al., 2003). Additionally, two weeks after completing a course, we found that BP ECS resulted in a significantly greater increase in hippocampal levels of BDNF than UBP ECS. Increased BDNF expression has been reported to mediate anti-depressant effects for a range of antidepressant drugs as well as ECS in animals (Castren and Rantamaki, 2010).

Chronic UBP ECS was therefore not as efficient as BP ECS for inducing antidepressant-related cellular and molecular changes in rat hippocampus. In parallel with these differences, we found that BP ECS had a greater antidepressant behavioural effect as it significantly reduced immobility time in the forced swim test, while immobility time following UBP ECS did not differ significantly from sham ECS. This may be reflected in some recent clinical studies of various forms of UBP ECT for depression that reported very low remission rates at only 6-13% (Loo et al., 2007; Quante et al., 2011).

A potential benefit of UBP ECS – and UBP ECT for treating depression – is a reduction in the cognitive side effects associated with ECS treatment (Sackeim et al., 2008). In the present study there was a non-significant trend for similar decreases in performance in both the UBP and BP groups compared to the sham control group on hippocampal-dependant reference memory in the water plus maze probe trial. However, we did not find any difference between the effects of UBP and BP ECS even though it induced less cellular, molecular and behavioural changes than BP ECS. These cognitive findings should be interpreted with some caution because the sample sizes in our study may have been too small to detect a relatively reduced impact of UBP ECS on memory. Additionally, the interval of 10 days between end of treatment and cognitive assessment may have led to early post treatment differences between UBP and BP groups no longer being apparent, reflecting recovery of hippocampal cognitive function over time following ECS as occurs in patients treated with ECT (Semkovska et al., 2011; Semkovska and McLoughlin, 2010). Interestingly, some recent studies attempting to cause neuronal loss using ECS have found that repeated brief seizures can cause specific neuronal death within hippocampal structures under certain conditions (Cardoso et al., 2011). However, these same studies also report that cell loss does not occur when ECS is administered on a 24-48 hour schedule (Cardoso et al., 2008). The interval between the final seizures in the programme must be short (2 hours) to cause the morphological and behavioural changes observed, possibly resulting from neuronal vulnerability during the post ictal period. Of note, analyses of rodent brain following conventionally spaced BP ECS, i.e. not designed to deliberately induce neuronal death, have not found evidence of neuronal loss (Dalby et al., 1996; Gombos et al., 1999; Vaidya et al., 1999; Cardoso et al., 2008; Jinno and Kosaka, 2009). Based upon the findings of the present study, we would predict the same for UBP ECS.

For experimental purposes we altered only one parameter in the treating ECS stimulus - the pulse width. Therefore, one possible explanation for the relatively small antidepressant-related effects we found with UBP ECS is that the total stimulus charge per session administered was only 60% that for BP ECS, i.e. 1.575 mC compared to 2.625 mC. Increasing the frequency of UBP stimuli or the duration of UBP ECS administration might result in greater anti-depressant effects. However, despite the lower total charge for UBP ECS used in the present study, both forms of ECS induced motor seizures that were

indistinguishable. Even though we found large and meaningful differences between UBP and BP ECS on several different measures, some caution is warranted as noted above because of the relatively small sample sizes and the risk of type 1 error. Our findings therefore require further exploration and replication, possibly examining reported differences at other timepoints. Finally, the effects of UBP ECS on animal models of depression, in addition to normal rats, need to be studied. For example, BP ECS has been reported to reduce forced swim test immobility time in Flinders Sensitive Line rats (Jimenez-Vasquez et al., 2007), normalise both impaired sucrose preference (a measure for anhedonia) and reduced hippocampal BDNF levels induced by chronic mild stress (Gersner et al., 2010), and reverse corticosterone-mediated inhibition of neurogenesis (Hellsten et al., 2002). It would therefore be important to study the therapeutic effects of UBP ECS in similar models of depression.

5. Conclusions

We found UBP ECS to be much less efficient than BP ECS in normal rats for inducing a range of cellular, molecular and behavioural changes that are believed to be important for antidepressant mechanisms. Comparing the effects of different stimulus parameters of ECS in animals allows us to better understand this antidepressant therapy and determine whether modifying the existing parameters of ECT may be of benefit for patients with depression.

Acknowledgements

This work was supported by the Health Research Board (grant no. TRA/2007/5) and the St. Patrick's Hospital Foundation.

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Legends for figures

Figure 1 Experimental design. (A) Animals were administered real or sham ECS thrice weekly for ten sessions over 22 days. Following one week habituation to the behavioural suite, animals were trained and tested in the water plus maze (WPM) followed by the forced swim test (FST) two days later. The animals were sacrificed 24 hours after the swim test. (B) Another set of animals was similarly treated with ECS and was also injected with BrdU 2-4 hours after ECS treatments 4-10. The animals were perfused 24 hours after the final ECS session and the brains extracted to examine changes in cell proliferation.

Figure 2 Seizure durations. (A) Tonic and (B) clonic seizure durations are shown on the first, fifth and tenth treatment days of the randomly allocated ECS course. Durations of both tonic ($p=0.038$) and clonic ($p<0.0001$) seizure phases increased significantly over time in both BP and UBP groups but there was no significant difference between treatment groups or significant Treatment x Time interactions. $n=6$ per group.

Figure 3 Water plus maze. (A) After completing the randomly allocated course of ECS, animals obtained the platform more frequently over successive training days 1-3 ($p=0.024$). Treatment type had no effect on number of platform acquisitions. $n = 5/6$ per group. (B) The number of platform acquisitions on probe test day 4 did not differ significantly between groups. BP and UBP groups obtained the platform less frequently than the sham group although this difference was not significant.

Figure 4 Forced swim test following ECS. BP animals spent significantly less time immobile than UBP or control animals. The decrease in immobility times in the UBP group was not significantly lower than in controls. $n=6$ per group, $*p<0.05$ compared to control group.

Figure 5 BDNF protein levels in the hippocampus following ECS. BDNF was significantly increased in the BP group compared to the control and UBP ECS groups. The increase seen in BDNF levels in the UBP group was not significantly higher than in the control group. $n=6$ per group, $*p<0.05$, $**p<0.01$ compared to control and UBP groups.

Figure 6 Cell proliferation following ECS. BP treatment significantly increased the relative number of BrdU-labelled cells in the dentate gyrus compared to sham-treated control animals ($p<0.05$) (percentage of control). The increase in BrdU-labelled cells was not significantly higher in the UBP group than in controls. $n=4$ per group, $*p<0.05$.

A

B

Figure 1

A

B

Figure 2

A

B

Figure 3

Figure 4

Figure 5

Figure 6

Highlights

- Ultrabrief pulse electroconvulsive therapy may be as effective as brief pulse ECT
- We compared ultrabrief and brief pulse electroconvulsive stimulation (ECS) in rats
- We examined molecular, cellular and behavioural antidepressant-related changes
- Both forms of ECS elicited similar types and durations of seizures
- Antidepressant-related changes were more efficiently induced by brief pulse ECS