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Muhammad Rafiq
INSERM U666, CHU de Strasbourg, France.

Zahid Mahmood
University of Management and Technology, Lahore, Pakistan

Sajed Ali
University of Management and Technology, Sialkot Campus, Sialkot, Pakistan

Laure Pain
INSERM U666, CHU de Strasbourg, France.

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Muhammad Rafiq[1, 2], Zahid Mahmood[2], Sajed Ali[3], Laure Pain[1]
[1]INSERM U666, CHU de Strasbourg, France.
[2]Institute of Clinical Psychology, University of Management and Technology, Lahore, Pakistan
[3]School of Sciences, University of Management and Technology, Sialkot Campus, Sialkot, Pakistan

Corresponding to: *Dr Muhammad Rafiq, Institute of Clinical Psychology, University of Management and Technology, Lahore Email: rafiqdar@hotmail.com / muhammad.rafiq@umt.edu.pk*

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ABSTRACT:
Brain Derived Neurotrophic Factor (BDNF) is a brain protein implicated in learning, memory and other cognitive functions. Changes in cellular brain functions as well as cognitive defects have been observed the days following anaesthesia, even for short-duration anaesthesia with/without surgery. Despites the role of neurotrophic factors in cognition, no data are still available on brain effects after anaesthesia. **Purpose:** To study the effect of minor surgery under short duration anaesthesia on cognition by investigating BDNF levels in plasma, hippocampus and cortex.
METHODS: Male rats received an intra-peritoneal injection of either 120 mg/kg of propofol or intralipids solution or minor surgery was performed under propofol anaesthesia. The animals were euthanized at ZT5 (peak of the circadian profile of brain BDNF in rat) after 3 days and brain homogenates of prefrontal Cortex and Hippocampus were prepared and blood was also collected for plasma. The amount of BDNF was assessed using ELISA (Millipore) on supernatant.
RESULTS: We observed an increase of the BDNF content in the brain supernatants when animals were submitted to propofol anaesthesia alone or to surgery under propofol anaesthesia, in cortex and in hippocampus samples. Propofol anaesthesia or surgery under anaesthesia differed significantly from the Control group, in both cortical (all $P<0.01$) and hippocampal structures (all $P<0.05$). For the cortical structure, we observed a marginal difference ($P=0.0590$) between animals submitted to propofol anaesthesia and surgery under propofol anaesthesia. One way analysis of variance revealed no significant effect of the procedure on plasmatic BDNF.
CONCLUSIONS: We observed for the first time that short-duration propofol anaesthesia induced a transient increase of BDNF expression at day 3 after anaesthesia and no significant effect of minor surgery under propofol anaesthesia. This shows that there are cognitive effects even after day 3 of anaesthesia and surgery.
KEYWORDS: Anaesthesia, propofol, surgery, bdnf, cognition, memory, learning.

INTRODUCTION

Brain Derived Neurotrophic Factor (BDNF) is known to support neuronal survival, differentiation and several forms of synaptic plasticity, therefore BDNF plays an important role in synaptogenesis of the mammalian brain(1). Synthesis, secretion and actions ofBDNF are largely mediated by neuronal network activity and in turn, activity dependent secretion of BDNF caninduce both rapid and long-term changes in synaptic efficacy(2). In this field, both decrease and overexpression of BDNF in the forebrain might have a detrimental effects on learning and memory in rodents (3).
Recent studies have evidenced that plasma BDNF concentrations werereduced 24 hours after lumber or cervical discotomy under general anaesthesia in male (35-65 years old) patients (4). It was also noted that plasma BDNF was decreased 15 minutes after the induction of anaesthesia and before the beginning of surgery procedure. Surgery decreases hippocampal BDNF 24 hours after tibia fracture performed under general anaesthesia in mice (5). The above results have led to suggest that surgery is responsible for post-operative learning impairment by acting on BDNF biosynthesis via the inflammation process linked to surgery. However, surgery was always performed under anaesthesia, and the proper effect of anaesthesia itself has not been clearly examined so far.
So, our objectives of the study were; to observe any significant effect between minor surgery under propofol anaesthesia and propofol alone on BDNF protein. To observe any significant effect of treatments between brain structures and plasma. For this, we used a short duration (30 min) anaesthesia (propofol intraperitoneally), a minor surgery procedure (mini laparotomy) in adult rats and we measured BDNF protein in plasma and tissue extracts removed

at the third post-operative day. The examination of brain BDNF was made at the same zeitgeber time (ZT5) for each animal to avoid any bias linked to circadian variability of BDNF.

MATERIAL AND METHODS

Experiments: All the experiments were conducted at Institute of Cellular and Integrative Neurosciences, Strasbourg France. All experiments were carried out in compliance with national (Council directive 87848, 19 October 1987, Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale;) and international guidelines (NIH publication, no. 86-23, revised 1985).

*Animals:*Young male Sprague Dawley rats obtained from Charles River were housed four to five rats per cage in a temperature (20 ± 1C) and humidity (40 ± 2%) on a twelve-hour light/dark cycle (lights on 7:00am), with *ad libitum* access to food and water.

*Treatment and sampling:*Animals were divided into three groups on the basis of treatment: propofol anaesthesia (*Fresenius, France*), minor surgery under anaesthesia and intra-lipid control (*Fresenius, France*).Depth of anaesthesia was assessed by the loss of righting reflex and toe pinch. Treatments were given at ZT10 (ten hours after the light on) and subjects were sacrificed at ZT5 after euthanasia under CO_2.Brains were quickly removed on ice and cortex and hippocampal structures were taken. Cortex and hippocampal tissues were homogenised in extraction buffer (containing Tris HCL 50mM, $MgCl_2$5mM, DDT 1mM, PMSF 0.5mM, EDTA 0.1 mM, EGTA 0.1mM and NaCl 0.9%). After centrifugation (4000 rmp, 20min), supernatants of the cortex and hippocampus were separated and stored at -20 C° for quantification of BDNF by ELIZA.

BDNF protein quantification: We also determined total protein contents by protein assay developed by (Bio-Rad) before BDNF quantification by ELIZA. Further, BDNF protein was quantified using an assay that is validated for both rat and human (CYT306, Millipore) according to manufacturer's recommendations (6-8).

Statistical Analysis:Two way analysis of variance was used to show any significant difference (between factor: Treatment; within factor: brain structures) by using SYSTAT. Post hoc analysis through Bonferroni was also done to observe any significant effect in experimental and control groups.

RESULTS

We observed an increase of the BDNF content in the brain supernatants when animals were submitted to propofol anaesthesia alone or to surgery under propofol anaesthesia, in cortex and in hippocampus samples.

Brain structures: Two way analysis of variance (between factor : treatment and within factor : brain structures) showed a significant effect of treatment (control, propofol and surgery under propofol); F(2,21) = 9.86 ; P=0.001) but no significant effect of the brain structure (cortex versus hippocampus; F(1,21)= 2.28) and no significant interaction between treatment and structure (F(2,21)=2.82) on the BDNF content in supernatant. Post-hoc analysis showed that animals submitted to propofol anaesthesia or surgery under anaesthesia differed significantly from the control group, in both cortical (all P<0.01) and hippocampal structures (all P<0.05). For the cortical structure, we observed a marginal difference (P=0.0590) between animals submitted to propofol anaesthesia and surgery under propofol anaesthesia. (Fig. 1A and 1B)

Blood plasma: The concentration of BDNF in plasma was unchanged whatever the treatment given; anaesthesia or surgery under anaesthesia (Fig 1C). One way analysis of variance revealed no significant effect of the procedure on plasmatic BDNF (F (2, 21) =0.27).

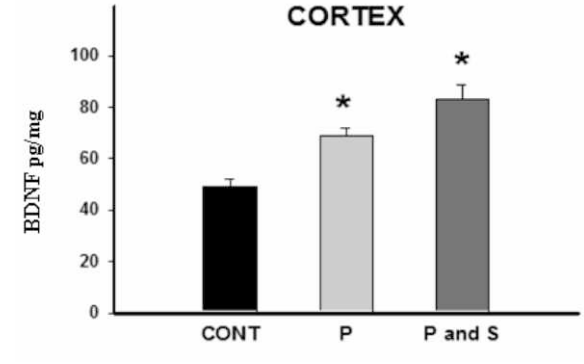

Fig.1A:
BDNF (pg/mg) observed at day 3 in rats' cortex supernatant treated with minor surgery under propofol anaesthesia, propofol anaesthesia alone and with intralipid as control.

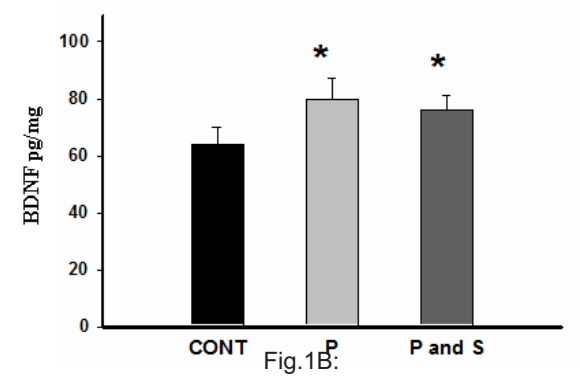

Fig.1B:
BDNF (pg/mg) observed at day3 in rat's hippocampus supernatant treated with minor surgery under propofol anaesthesia, propofol anaesthesia alone and with intralipid as control.

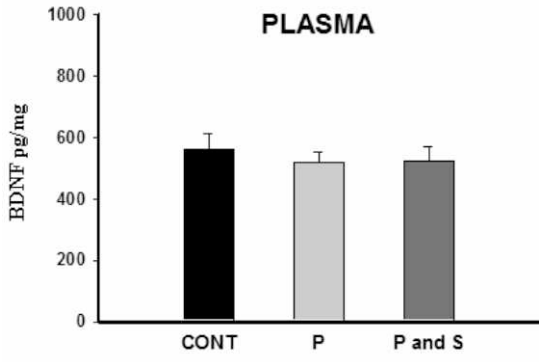

Fig.1C:
BDNF (pg/ml) observed at day3 in rat's plasma treated with minor surgery under propofol anaesthesia, propofol anaesthesia alone and with intralipid as control.

DISCUSSION

In the present study, we observed the effect of minor surgery under short duration propofol anaesthesia and propofol anaesthesia alone on brain and plasma protein after 3 days of treatment. We observed an increase of BDNF protein in the cortex and hippocampal supernatants when subjects were submitted to propofol and we did not observe any increase of BDNF protein in both the structures with minor surgery. We also determined that there is no significant modification in BDNF protein in the plasma. The BDNF protein in the cortex and hippocampal supernatants and plasma was determined with ELISA technique. ELISA is a quick and convenient method for the determination of antigens in tissue homogenates and in blood. This has been shown to achieve sensitivities and specificities (9, 10).

Some data obtained on the effect of general anaesthetics on the developing brain demonstrated that anaesthesia alone may impact in a complex manner on the biosynthesis of BDNF. General anaesthetics triggered a down regulation of BDNF protein in thalamusand an up regulation in cerebral cortex in pups (11). This study was conducted on 7-day old Sprague-Dawley rat pups as this age is at the peak of their brain developmentwhen they are most vulnerable to anaesthesia-induced apoptotic damage(12). Vutskits et al. (2008) evidenced that plasma BDNF concentrations wasreduced 24 hours after lumber or cervical discotomy under general anaesthesia in male patients.Fidalgo et al. (2011) also noted that plasma BDNF was decreased 15 minutes after the induction of anaesthesia and before the beginning of surgery procedure and surgery has decreased hippocampal BDNF 24 hours after tibia fracture performed under general anaesthesia in mice. These studies have demonstrated the immediate effect of either anaesthesia or surgery. These studies are performed without mentioning any specific circadian or zeitgeber time, as it has been evidenced that protein vary rhythmically during 24hours of the day. Our study is unique in two ways, determining effect of propofol anaesthesia at day 3 and at zeitgeber time 5 (ZT5), that is five hours after the light on. It is important to study the protein expression at specific time, as it has been studied that suprachiasmatic nucleus synchronises different circadian clocks throughout the body according to different environmental zeitgebers (13).

Our data for the first time evidenced that brain BDNF in the cortex and hippocampus increases at day 3 of anaesthesia. This post anaesthesia increase of BDNF may be due to its daily rhythms, as BDNF is observed at peak in rodent's brain at ZT5 in our unpublished results. In this field, both decrease and overexpression of BDNF in the forebrain might have a detrimental effect on learning in rodents (3). However, we did not find any significant effect of minor surgery on BDNF protein. This may be due to that minor surgery is not able to induce sufficient inflammation to alter BDNF protein. A study also show that minor surgery is not able to induce sufficient stress response to alter proteins (14). Our data also shows no significant alteration of BDNF protein in the plasma in either propofol or surgery treated subjects with their intralipid control.

Studies have evidenced that anaesthetics either short duration or long duration have been implicated in the depression of neuronal activity (15)and Lu (2003) evidenced that processing and functioning of BDNF mainly depend on the neuronal activity and in this way, synaptic plasticity, learning, memory and congition depend on activity of the nervous system and secretion of BDNF. So, anaesthetics are responsible for alternation of BDNF by the depression of neural activity and detrimental effects on neuronal structures. In response to detrimental effects of anaesthetics, BDNF may increase to overcome the damage as our results demonstrated. Bibel and Barde (2000) studied that BDNF plays very important role in synaptic plasticity which is further responsible for learning and memory performance. So it important to assess the cognitive abilities even after days of anaesthesia and or surgery and management strategies should be adopted to enhance and manage the cognitive abilities especially in patients with multiple surgeries. As our results indicate alteration of BDNF at day 3 after anaesthesia but studies have evidenced that protein modification exist even after weeks. At least, this needs to be determined, for this it will be necessary to observe long term effect of anaesthesia on memory performance.

As the surgery we performed here was minor with no major inflammation reaction under short duration anaesthetics. From our results, we cannot preclude a combined effect of short duration anaesthesia and major surgery on brain BDNF protein. Our study also cannot mimic results on long duration anaesthetics as well. We performed our experiment at certain zeitgeber time (ZT5), five hour after light on period, so we cannot

conclude this alteration of protein at other circadian times, as proteins vary during different time of the day. This should be determined at least six different times during 24hours of the day. This will help to find out the circadian time where there will be minimum detrimental effects of anaesthetics on the brain. This will further help anaesthesiologists to inject anaesthesia at a time where minimum damage, a chrono-therapeutic approach (16). This study will also build foundation for further studies including application of cognitive therapies in the management of post-anaesthetics cognitive impairments.

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Author's contribution:
Muhammad Rafiq; concept, data collection, data analysis, manuscript writing, manuscript review
Zahid Mahmood;data collection, data analysis, manuscript writing, manuscript review
Dr. Sajed Ali; Manuscript writing and review
Laurie Pain; concept, data analysis, manuscript writing, manuscript review
Dr Zahid Mahmood; Manuscript editing and review