LONG-TERM EFFECTS OF FENTANYL ON THE BREATHING OF NEW-BORN MICE

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ABSTRACT

Abnormal breathing control is linked to several disorders such as sleep apnoea, panic attack, and sudden infant death. This study investigated the development and functioning of breathing control. Previous studies found that a small part of the brain, the preBötzinger Complex, is responsible for the generation of automatic, rhythmic breathing. The preBötzinger Complex is still developing during the first few days after birth, making this period extremely vulnerable to drugs. One of the first painkillers offered to mothers during child-birth is an opioid painkiller known as fentanyl, but its effects on new-borns are not entirely understood. Previous research shows that fentanyl can cross the placenta and it can be secreted into breast-milk. In this study, new-born mice were injected with fentanyl to test its long-term effects on breathing. These animals were also injected with fentanyl during adulthood to test whether they respond to the painkiller differently to those animals that have never been exposed. The fentanyl pre-exposed mice showed a decreased baseline breathing, an altered response to fentanyl during adulthood, and increased number of sighs, hiccups, apnoea and hyperpnoea. Further findings showed increased weight and abnormal stress-related behaviour in these mice. These results suggest that fentanyl exposure can have long-term effects on new-borns. The findings of the present study raise the question of the safety of maternal fentanyl use during pregnancy, labour and breastfeeding.

INTRODUCTION

Breathing is a never-ceasing activity of all mammals, including humans. It enables us to exchange oxygen and carbon-dioxide in order to maintain the metabolism that keeps us alive. A lack of oxygen, called hypoxia, during the early developmental stages can lead to several complications in later life (Cooper, 2015). Thus, it is very important to understand how breathing is controlled.

There are several disorders that are linked to abnormal control of breathing, but their exact causes are still unknown. Sleep apnoea is one of the most well-known disorders of all (Eckert et al., 2007). People who suffer from sleep apnoea tend to stop breathing during sleep for an abnormal period of time (more than 10 seconds). Longer breaks in breathing can lead to extreme hypoxia, causing awakening that will then re-establish a normal breathing rhythm. Some people are not aware of their disorder as they cannot recall waking up, but the disturbed sleeping pattern and prolonged hypoxia can have detrimental effects on health. For instance, a lack of normal sleeping pattern has been associated with the risk of developing Alzheimer's disease (Macedo et al., 2017).

Anxiety and panic attacks have also been associated with an abnormal control of breathing (Ramirez, 2014). Panic attacks are characterised as periods of hyperventilation triggered by stressful situations, and many people experience them during their lifetime. Anaesthesia-induced respiratory arrest is another serious cause of breathing abnormality (Tobias and Leder, 2011). Patients undergoing surgery are often anesthetized, and some stop breathing unexpectedly during or after surgery. This could lead to death when these patents are left unattended. Finally, sudden infant death is another little-understood condition where new-borns stop breathing during sleep and die without any apparent warning signs (Garcia, Koschnitzky and Ramirez, 2013).

In order to understand these disorders, it is important to understand the exact mechanism of breathing. Neuroscientists have been studying the control of breathing over several decades. Respiratory control is much more complex than it seems. Breathing can be controlled voluntarily via complex pathways that originate in the primary motor cortex of the brain. That is how we are able to speak, sing and dive under water. But under normal circumstances, breathing is under automatic control. That is why we continue to breath rhythmically during unconscious states such as sleep and coma. Automatic breathing is controlled by a small area of the brain, known as the preBötzinger Complex (Smith et al., 1991) (Figure 1).

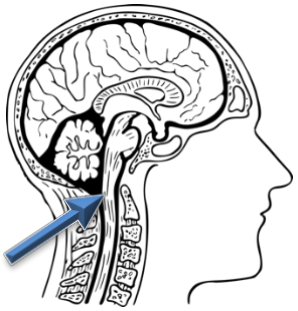

Figure 1: The preBötzinger Complex is the centre of automatic breathing control, and is situated in the brainstem.

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The preBötzinger Complex is a group of brainstem neurons that have rhythmic activity (Feldman et al., 2013). This rhythmic neural activity drives rhythmic contraction of the respiratory muscles such as the diaphragm and the intercostal muscles. Contraction of these muscles causes inhalation, while relaxation of these muscles leads to exhalation.

The preBötzinger Complex is an important area of interest in the field of Neuroscience, as its exact functioning is still not entirely understood. Neuroscientists have therefore been investigating the development of the preBötzinger Complex to understand its role in respiratory control. It is believed that the preBötzinger Complex continues developing during the first few days after birth (te Pas et al., 2009). This immature functioning can also be observed in new-borns as they present arrhythmic breathing. During this developmental period, the brain is highly vulnerable to external perturbations. For instance, drugs can interfere with the normal development of the brain causing long-term changes in its functioning.

New-borns can be indirectly exposed to drugs during pregnancy, birth and breast-feeding. One of the most common drugs that mothers receive during these periods are opioid painkillers. Fentanyl is a widely used opioid painkiller that can cause pain-relief, sedation, euphoria and addiction (Tharp et al., 2004). Fentanyl is an extremely strong drug. It is a thousand times stronger than heroin, and hence even a small dose can be lethal. Fentanyl is also used recreationally, with many cases leading to death, such as the case of the famous singer, Prince, who recently died from a fentanyl overdose. Fentanyl can lead to death caused by respiratory arrest due to its depressing effect on the preBötzinger Complex. Several clinical cases have reported that maternal fentanyl use caused depressed breathing in new-borns and other birth complications (Briggs et al., 2005). The long-term effects of fentanyl on breathing however, have not yet been investigated.

METHODS

The present research aimed to investigate the long-term effect of fentanyl exposure on the breathing of new-born mice (Figure 2). After birth, 15 mice were divided into two groups. Six mice received daily injections of 0.04 mg/kg dose of fentanyl for five days while the control group received saline injections that are known to have no physiological effect. Four months later, the resting breathing pattern of the now-adult mice was recorded to see whether new-born fentanyl exposure had a long-term effect on breathing. Following the recordings, the second part of the experiment began. At this point, both the fentanyl pre-exposed and the control groups were injected with a single dose of 0.06 mg/kg fentanyl to monitor their response to the drug. To be able to analyse the response, recording of the breathing pattern was repeated after the injections.

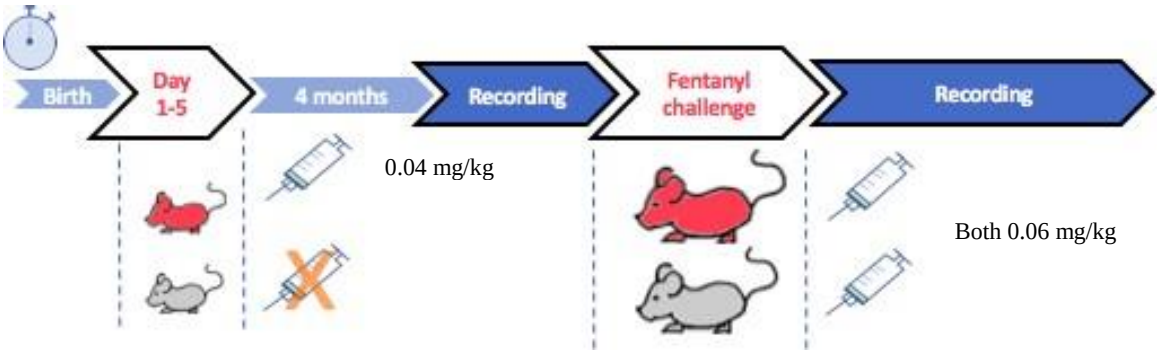

Figure 2: Experimental timeline. Red mice represent the fentanyl pre-exposed group, and grey mice represent the control group. Syringes represent fentanyl injections. Recording of ventilation was carried out using plethysmography.

Breathing patterns were measured using plethysmography, which is a non-invasive technique (Drorbaugh and Fenn, 1955). The plethysmograph consists of two boxes (Figure 3). Mice are put in the sealed recording chamber where they breathe in and out, creating a difference in pressure. This fluctuation in pressure is compared to the static pressure in the reference chamber by a differential pressure transducer. These signals are then forwarded to a computer where the breathing pattern is presented as waves; each wave represents one breath. A breath-by-breath analysis was then caried out using Spike 2 software to quantify breathing.

Additionally, the breathing pattern was manually analysed for breathing artefacts such as apnoeas, hyperpnoeas (increased frequency of breathing), hiccups (quick inhalation and exhalation) and sighs (high volume inhalation and slow exhalation).

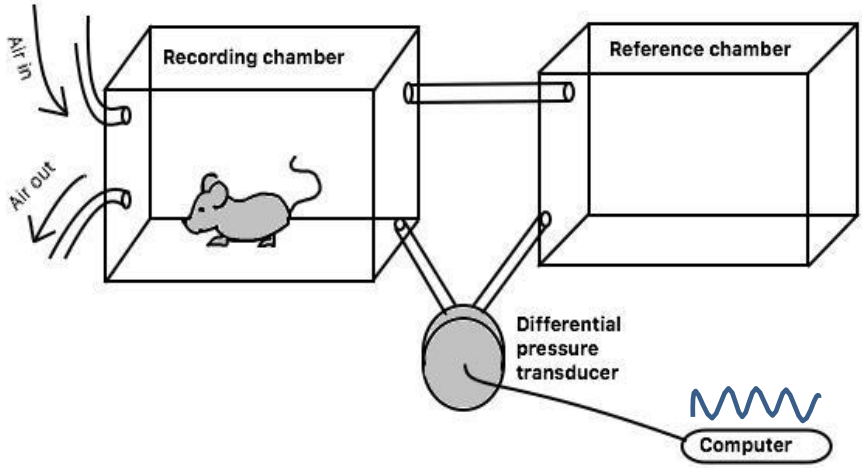

Figure 3: The plethysmograph. Plethysmography is a non-invasive way of measuring ventilation (Drorbaugh and Fenn, 1955). The differential pressure transducer measures and compares the pressure changes in the recording chamber to the reference chamber as mice breath in and out. Single breaths are represented as single waves on the computer.

RESULTS

The results of the present study indicate multiple differences between the fentanyl pre-exposed and the control groups.

Firstly, the fentanyl pre-exposed group was found to have a depressed baseline ventilation compared to the control group (Figure 4; fentanyl pre-exposed group: 0.03 ± 0.001 ml/min/g, *vs* control group: 0.04 ± 0.004 ml/min/g).

Secondly, fentanyl injection in adulthood resulted in different responses in the two groups. The control group showed an expected depression in ventilation after fentanyl injection, while the fentanyl pre-exposed group showed an increase in ventilation five minutes after the fentanyl injection that was followed by a slow return to baseline ventilation (Figure 4). Although statistical analysis showed no significance, probably due to the small sample size, the raw data suggest a long-term effect of early fentanyl-exposure on breathing control.

Further analysis of the breathing patterns reveal that the fentanyl pre-exposed mice tend to present more apnoeas, hyperpnoeas, hiccups and sighs.

Finally, other non-respiratory related group differences were observed. The fentanyl pre-exposed group showed increased weight and increased stress-related behaviour, such as restlessness, altered social interaction and an increased monitoring of the environment.

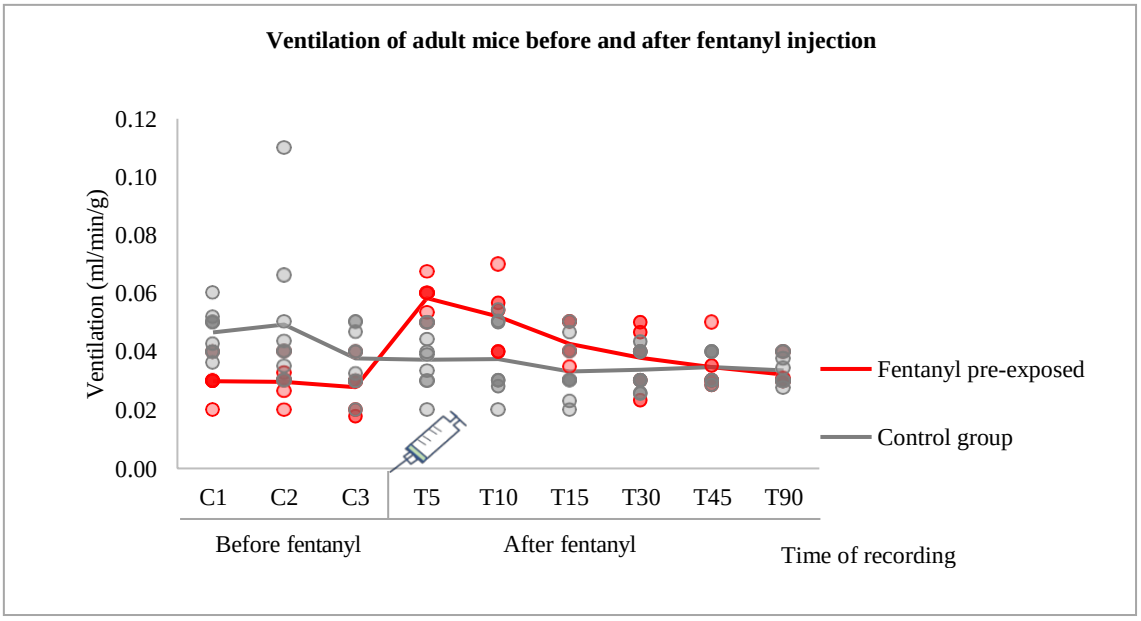

Figure 4: Ventilation before and after fentanyl injection in adult fentanyl pre-exposed and control mice. Single points represent individual animals and lines represent the average ventilation amongst each group. C1-3 represent three control recordings before fentanyl injection where fentanyl pre-exposed mice showed depressed baseline ventilation compared to controls. T5-T90 represent six time-points in minutes after fentanyl injection when plethysmography recording was carried out. Fentanyl pre-exposed mice showed increased ventilation, while the control group showed depressed ventilation after fentanyl injection.

DISCUSSION

The findings of this study show that fentanyl exposure can have long-term effects on new-borns. It is difficult to interpret these results, however, due to a lack of research in this area. It is postulated that the depressed baseline ventilation is due to a functional change in the respiratory centre of the brain. Early fentanyl exposure could lead to long-term changes in the activity of the preBötzinger Complex, which may be due to an altered number of cells, connections, and/or their signalling.

As there was a different response to fentanyl injection between groups during adulthood, it is thought that the fentanyl pre-exposed animals developed a tolerance towards opioids (Williams al., 2013). In the case of opioid-tolerance, only a higher dose of fentanyl would be able to produce similar results to the control group. Tolerance does not, however, explain the initial increase of ventilation in the pre-exposed animals after fentanyl injection in adulthood.

The sudden increase in ventilation after a fentanyl injection could be an acute response to the experimental procedure itself rather than the opioid. Previous studies suggest that opioid pre-exposure can lead to increased pain sensation (Hayhurst and Durieux, 2016). Fentanyl is administered via systemic injections, and increased pain sensation could result in elevated stress response, hence higher blood pressure and increased ventilation. Additionally, opioid pre-exposure could interfere with the development of the hypothalamic-pituitary-adrenal axis that is responsible for normal stress response (Vuong et al., 2010). This could also serve as an explanation for the observed stress-related behavioural changes in the fentanyl pre-exposed animals.

Finally, it is postulated that due to the depressed activity of the preBötzinger Complex, other respiratory areas in the brain, such as the retrotrapezoid nucleus, might overtake the control of respiration, leading to a change in ventilation (Onimaru and Homma, 2008). As studies indicate that breathing control is state-dependent, a change in respiratory control areas can also increase the risk of complications during depressed brain states such as sleep (Doi and Ramirez, 2010).

Future studies are needed to further investigate the diverse physiological and psychological effects of early fentanyl exposure. Repetition of the present study with a larger sample size is needed to earn realistic statistical significance. Additionally, structural and compositional analysis of the preBötzinger Complex of both experimental groups are needed, to ascertain the mode of action of fentanyl on respiratory control.

CONCLUSION

The results of the present study show that exposure of new-born mice to fentanyl can cause a depressed baseline ventilation in the long-term and can induce a different response to fentanyl exposure during adulthood. Additionally, fentanyl exposure of new-born mice was associated with other long-term physiological and psychological changes such as increased weight and increased stress-related behaviour. Although these results are only the first steps towards understanding the long-term effects of opioid painkillers on new-borns, these findings question the safety of maternal fentanyl use during pregnancy, labour and breast-feeding. While scientists are working hard to find the answers on drug safety during these periods, it is important to raise public awareness of the possible risks.

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REFERENCES

Briggs, G.G., Freeman, R.K., and Yaffe, S.J. (2005). Drugs in pregnancy and lactation: a reference guide to fetal and neonatal risk (Philadelphia, Pa: Lippincott Williams & Wilkins).

Cooper, J. M. et al. (2015) Neonatal hypoxia, hippocampal atrophy, and memory impairment: evidence of a causal sequence. Cerebral Cortex, 25(6), pp. 1469-1476.

Doi, A. and Ramirez, J. (2010). State-Dependent Interactions between Excitatory Neuromodulators in the Neuronal Control of Breathing. *Journal of Neuroscience*, 30(24), pp.8251-8262.

Drorbaugh, J.E., and Fenn, W.O. (1955). A barometric

method for measuring ventilation in newborn infants.

Paediatrics 16(1), 81-7. Eckert, D., Jordan, A., Merchia,

P., and Malhotra, A. (2007). Central sleep apnea. Chest

131(2), 595-607.

Feldman, J., Del Negro, C., and Gray, P. (2013). Understanding the rhythm of breathing: So near, yet so far. Annual Review of Physiology 75(1), 423-452.

Garcia, A., Koschnitzky, J. and Ramirez, J. (2013). The physiological determinants of Sudden Infant Death Syndrome. Respiratory Physiology & Neurobiology, 189(2), pp.288-300.

Hayhurst, C., and Durieux, M. (2016). Differential opioid tolerance and opioid-induced hyperalgesia. Anesthesiology 124(2), 483-488.

Macedo, A., Balouch, S. and Tabet, N. (2017). Is Sleep Disruption a Risk Factor for Alzheimer's Disease?. *Journal of Alzheimer's Disease*, 58(4), pp.993-1002.

Onimaru, H., and Homma, I. (2008). Two modes of respiratory rhythm generation in the newborn rat brainstem- spinal cord preparation. Adv. Exp. Med. Biol. 605, 104-108.

Ramirez, J. (2014). The integrative role of the sigh in psychology, physiology, pathology, and neurobiology. Progress in Brain Research, 91-129.

Smith, J. C., Ellenberger, H. H., Ballanyi, K., Richter, D. W., and Feldman, J. L. (1991). Pre- Bötzinger complex: A brainstem region that may generate respiratory rhythm in mammals. Science 254(5032), 726–729.

te Pas, A., Wong, C., Kamlin, C., Dawson, J., Morley, C., and Davis, P. (2009). Breathing Patterns in Preterm and Term Infants Immediately After Birth. Pediatric Research 65(3), 352-356.

Tharp, A.M., Winecker R.E., and Winston, D.C. (2004). Fatal intravenous fentanyl abuse: Four cases involving extraction of fentanyl from transdermal patches. Am. J. Forensic. Med. Pathol. 25(2), 178-181.

Tobias, J., and Leder, M. (2011). Procedural sedation: A review of sedative agents, monitoring, and management of complications. Saudi Journal of Anaesthesia 5(4), 395.

Vuong, C., Van Uum, S., O'Dell, L., Lutfy, K., and Friedman, T. (2010). The Effects of Opioids and Opioid Analogs on Animal and Human Endocrine Systems. Endocrine Reviews 31(1), 98-132.

Williams, J., Ingram, S., Henderson, G., Chavkin, C., von Zastrow, M., Schulz, S., Koch, T., Evans, C., and Christie, M. (2013). Regulation of μ-Opioid Receptors: Desensitization, Phosphorylation, Internalization, and Tolerance. Pharmacological Reviews 65(1), 223-254.