Addis Ababa University

College of Health Science (Graduate Studies)

School of Medicine

Department of Medical Anatomy

Project Paper On: Histological and functional effect of nicotine on cerebral cortex of the brain

Project Paper Submitted to Addis Ababa University College of Health Sciences School of Medicine, Department of Medical Anatomy in the Fulfillment of the requirement for the Degree of Master of Science in Human Anatomy

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July 2016

Acknowledgement

First and foremost, I would like to thank my lord and savior Jesus Christ for his infinite grant throughout my life.

I would like to extend my deepest gratitude and appreciation to my advisor Dr. Girma Seyoum for his constructive advice and support throughout the development and completion of this project paper.

I extend my thanks to Addis Ababa University for sponsoring me to the postgraduate study.

I thank my friend Yemisrach Workneh and staff members of the department of Anatomy. Finally, my unique thanks and gratitude go to my families for their assistance throughout my study.

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List of Abbreviation

CA4……..Cornu Ammonis four

CO……Carbon monoxide

Co_2……..Carbon dioxide

CUN…….Cunate

DMNV……..Dorsal motor nucleus of vagus

DOPAC…….3, 4- Dihydroxy phenyl acetic acid

ECG……...Electrocardiograph

EDTA…….Ethylene-Diamine-Tetra-acetic Acid

FAD……Flavin Adenine Dinucleotide

FDA……Food drug administration

GD……..Gestational day

GRAC…….Gracile

HCL……Hydrochloric acid

HVA……Homovanilic acid

ION……. Inferior olivary nucleus

MHPG…….3-Methoxy-4hydroxypheylethyl ethylenglycol

NaCl……..Sodium chloride

NADPH……..Nicotinamide adenine dinucleotide phosphate

NaOH…….Sodium hydro oxide

NHT…….Nicotine hydrogen tartrate

NMN……Normetanephrine

NO……Nitric oxide

NSTT……Nucleus of spinal trigeminal tract

NTS…….Nucleus of the solitary tracts

O_2……Oxygen

PCo_2…….Plasma carbon dioxide

PET……...Postrion emitting tomography

PND……. Postnatal day

rCBF…….Regional cerebral blood flow

PPM……..Parts per million

SIDS…….Sudden infant death syndrome

TUNEL…….....Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling or (DNA fragmentation)

$ZnSO_4$……Zinc sulfate

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Summary

Nicotine is mainly found in different types of tobacco but it is also present in lower doses in different types of foods. This paper reviews the scientific literatures linking nicotine with its effect on histological and functional system of cerebral cortex of the brain. Studies were assessed by focusing on the dose of nicotine, duration of exposure, type of experimental animals and mechanism of exposing which were used to measure the effect of nicotine. The literatures reviewed in the present paper used human being, dogs, fetal and adolescent rats, and postnatal piglets as experimental animals. From the literatures reviewed in the present paper, nicotine showed to be neurotoxin alkaloid which affects the neurochemistry of brain, cause abnormalities in brain morphology, neurodegeneration, and histological alternation in hippocampus, cerebral cortex and in different parts of the brain.

Keywords: Nicotine, cerebral cortex, histophatological effect, neurochemistry

1. Introduction

1.1 Physical and Chemical Properties of Nicotine

Nicotine is an alkaloid found in the leaves of the tobacco plant (*Nicotiana*); cigarettes are manufactured from the two species *N. tabacum* and *N. rustica*. The first indications of tobacco use by humans (smoking of dried tobacco leaves in a clay pipe called a *Tobago* by American Indian tribes) were discovered in the 15th century, after the landing of Columbus and his crew in the Caribbean. In the 16th century, tobacco was already known in the Iberian Peninsula, and its Further spread through Europe was due to a French physician and diplomat, Jean Nicot de Villemain, who recommended the use of nicotine as medication. Trace amounts of nicotine are found also in potatoes, tomatoes and sweet pepper (Anderson *et al*., 2003). Nicotine content in tobacco leaves approximates 1.5 % of their dry weight. A cigarette contains 8.4 mg of nicotine on average, of which 1–3 mg is absorbed in humans through the inhalation of the smoke (Pogocki *et al*., 2007; US DHHS, 1988). Nicotine was isolated from tobacco in 1828; its structure was established 15 years later, and it was synthesized in 1904.

It is water-soluble, colorless, and bitter-tasting in the liquid form and is a weak base with pH 8.5. Nicotine is not to be confused with nicotinic acid, which is the fat-soluble vitamin B-3, called niacin, used in the treatment of pellagra, a niacin deficiency syndrome characterized by cutaneous, gastrointestinal, neurologic, and mental symptoms.

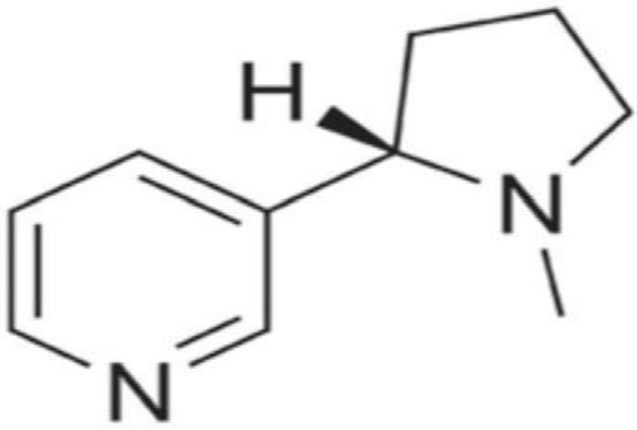

Figure 1.Structural formula of nicotine ((-)-1-methyl-2-(3-pyridyl) pyrrolidine;C10H14N2) (Source: Pogocki *et al*., 2007)

1.2. Occurrence and Sources of Nicotine

Nicotine is the principal tobacco alkaloid occurring to the extent of about 1.5% by weight in commercial cigarette tobacco and comprising of 95% of the total alkaloid content (Benzowit *et al*., 1983). Nicotine is also present in lower quantities in tomato, potato, eggplant (aubergine), green pepper and in the leaves of the coca plants (Martin, R. 1970).

1.3. Ways of Nicotine entrance into human body

Nicotine absorption can occur through the oral cavity, skin, lung, urinary bladder, and gastrointestinal tract (Schevelbein *et al*., 1973). The absorption of nicotine through the oral mucosa has been shown to be the principal route of absorption for smokers who do not inhale and for smokeless tobacco users. The principal route of nicotine absorption in smokers who inhale is through the alveoli of the lung. When tobacco smoke reaches the small airways and alveoli of the lung, the nicotine is rapidly absorbed. Blood concentration of nicotine rises quickly during the completion of cigarette smoking. The rapid absorption of nicotine from cigarette smoke through the lungs, presumably because of the huge surface area of the alveoli and small airways, and dissolution of nicotine in the fluid of pH 7.4 in the human lung, facilitates transfer across membranes. On average, around 1.0 mg (range 0.3–2–0 mg) of nicotine is absorbed systemically during smoking(Benzowit *et al*.,1984) from 80% to 90% of inhaled nicotine is absorbed as assessed using 14C-nicotine (Armitage *et al*.,1975).

Absorption through the alveoli is also dependent on the nicotine concentration in the smoke. Plasma nicotine levels depend on the type of cigarette smoked. In fact, there is much more adsorption with dark than with blond tobacco(Ferris et al.,2006) .Thus, Schevelbein *et al.,* 1973 shown that the plasma nicotine level in non-inhaling smokers is around 2.5–8.0 ng/ml, where as the plasma nicotine levels in inhaling smokers reach 30–40 ng/ml nicotine.

1.4. Major uses of nicotine

Nicotine, the addictive chemical in tobacco, is a pharmacotherapy for smoking cessation, and a useful probe drug for phenotyping cytochrome P450 2A6. It is used as an insecticide and fumigant, and forms salts with most acids (Hukkanen *et al.,* 2005).

1.5. Fate of Nicotine Absorbed by the Body

The smoke of cigarette contains several chemicals that are potentially toxic and carcinogenic to the human body, and many of these constituents have been linked to different diseased states. Cigarette smoking is a known risk factor of many clinical conditions, and can also exacerbate some conditions (Henderson, 2008; Jill *et al*., 2006; Tatum and Shapiro, 2005). Since many of these constituents, like nicotine, cross the blood-brain barrier, the brain has become a target also of the toxic effects associated with tobacco smoke.

Use of tobacco products during pregnancy has been related to some common neurobehavioral and cognitive outcomes in the offspring. Such features include increased externalizing behavior, decreased general cognitive function, and learning and memory deficits, among others (Huizink and Mulder, 2006).

1.6. Effect of Nicotine on Human Health

Use of tobacco products has been linked to abnormalities in brain morphology, neurochemistry, cerebral blood flow as well as neurocognition (Brody *et al.,* 2004). However, nicotine affects most organ systems in the body (Benowitz, 1996). Chronic smokers compared to non-smokers demonstrate lower cortical gray matter volumes and densities in the prefrontal cortex, smaller left anterior cingulated volume and lower gray matter densities in the right cerebellum (Brody *et al.,* 2004). Meanwhile, a study by Tanabe *et al*. (2005) revealed that nicotine may improve smooth pursuit eye movement performance in people with schizophrenia through cholinergic stimulation of the hippocampus and cingulated gyrus. A link exists between nicotine administration and brain circuitry that mediates visuospatial attentional processing and withdrawal symptoms (Brody, 2006). Prenatal nicotine exposure elevates risk of cognitive and auditory processing deficit and of smoking in offspring (Jacobsen *et al.*, 2007).

According to Thakur and co-workers (2013), maternal smoking during pregnancy is associated with a more severe form of attention deficit hyperactive disorder characterized by more severe clinical manifestations and poorer neuropsychological performance. When used at low doses, there is significant deregulation of transcription in placental and fetal cells (Votavova *et al.*, 2012).

Nicotine administration has been suggested to have beneficial effects in patients with hypoinsulinism (Hosseini, 2011). Although cigarette smoking has been associated with increased insulin resistance, this effect is not likely to be seen in healthy subjects (Xu *et al*., 2012).

2. Review and Analysis of Published Research Articles on the Histological and Functional Effects of nicotine in the Cerebral Cortex of the Brain

In this project paper, several investigations conducted by many researchers on the histological and functional effect of nicotine on cerebral cortex of the brain are reviewed. The methods used in the studies presented here are more or less similar. The animal models used in most of the studies reviewed here were dogs, fetal and adolescent rats, and postnatal piglets. In one study human subjects also were used. The published papers reviewed and analyzed are presented as follows.

A study was conducted by George *et al*., 1983 on the effect of nicotine on regional blood flow of cerebral cortex in dogs during intravenous infusion**.** In this study, thirty-three large, adult, mongrel dogs were used.

The dogs were anesthetized with sodium pentobarbital, 30 mg/kg initially and supplemented as required to maintain a stable plane of anesthesia. After tracheotomy and left thoracotomy, the animal was ventilated with room air (supplemented with 100% o_2 when necessary) to maintain physiological blood gases. A flow transducer was positioned on the ascending aorta, so that cardiac output (minus coronary blood flow) could be measured with an electromagnetic flow meter ,Micron RC1000(Micron Instruments, Inc., Los Angeles, CA). The flow transducer was calibrated *in vitro* on a segment of aorta Perfused, with whole blood. Mean aortic, central venous, and left atrial pressures were monitored through catheters connected to Statham pressure transducers, model P23Db (Gould Inc., Oxnard, CA) Limb lead II of the electrocardiogram was used to drive a cardiotachometer. A record of Blood pressures, aortic blood flow, electrocardiogram, and heart rate was made with a Beckman recorder, model R411 (Beckman Instruments, Inc., Schiller Park, IL). Succinylcholine (Anectine, 2 mg/kg i.v., supplemented as necessary; Burroughs Well come Co., Research Park, NC) was administered to prevent spontaneous respiratory movements during nicotine infusion. Heparin (500 U/kg i.v.) was used as an anticoagulant. Regional CBF values were determined from tissue content of 15±3μ microspheres administered into the left atrium. The microspheres were labeled with gamma-emitting radionuclides (^{46} Sc, ^{57}Co, ^{85}Sr, ^{113}Sn; New England Nuclear, Boston, MA, and 3M Co., St. Paul, MN). Prior to injection, the microspheres were dispersed by agitation in an ultrasonic bath and with a vortex mixer. Approximately 10^6 microspheres were administered for each flow determination.

Upon injection of microspheres, duplicate reference samples of arterial blood were withdrawn from two different sites in the aorta at a constant rate (approximately7.5 ml/min) for 2 min, so that regional blood flows could be computed.[18] Similarity of radioactivity in duplicate reference samples verified adequate mixing of microspheres. Each dog received two injections of differently labeled microspheres to measure Regional cerebral blood flow values under the pre-nicotine control condition and during intravenous infusion of nicotine. After the final dose of microspheres, the dog was killed by intravenous injection of potassium chloride. The skull was opened and the brain plus a cervical segment of the spinal cord were removed. Samples of tissue were cut from the frontal and occipital lobes of the cerebral cortex, the cerebellum, the pons, the medulla, and the spinal cord. These tissue samples and the reference arterial blood samples were analyzed for radioactivity in a gamma counter equipped with a multichannel analyzer (Packard Instrument Co., Downer Grove, IL).

The dogs were divided into two series: series I (effect of nicotine with uncontrolled Aortic pressure); and series II (effect of nicotine with constant Aortic pressure) and with four sub groups under series II.

Series I. Effect of nicotine with uncontrolled aortic pressure: In nine dogs control measurements of hemodynam-'ic parameters and of Regional cerebral blood flow were first obtained after sufficient time for stabilization of experimental preparation. These dogs, with intact autonomic receptors, then received intravenous infusion of nicotine (36 /µg/ kg/min). This rather high dose of nicotine was chosen throughout the study, so that hemodynamic responses were pronounced and modifications of these responses by autonomic blockers were readily detectable. Second injection of microspheres was made to measure again Regional cerebral blood flow values. The nicotine infusion was continued for two minutes after this injection of microspheres.

Series II. Effect of nicotine with constant aortic pressure: In order to eliminate the contribution of nicotine induced increases in aortic blood pressure to changes in Regional cerebral blood flow, studies were conducted in four groups of animals whose aortic pressure was held constant. Aortic pressure was controlled by connecting a 500 ml, pressurized reservoir bottle to the left subclavian artery, which had been ligated and then cannulated with wide-bore tubing. Reservoir pressure was maintained equal to mean aortic pressure with compressed gas. During infusion of nicotine, vascular constriction caused blood to be translocated from the dog's circulation to the

reservoir, allowing aortic blood pressure to be maintained within 5 mm Hg of the control blood pressure.

Group1. Without Autonomic Blockade in six dogs with intact autonomic receptors, control measurements of hemodynamic parameters and rCBF by the microsphere technique were first obtained. Nicotine was then infused and hemodynamic parameters were recorded and a second injection of microspheres was made at peak reservoir volume.

Group2. After Selective Beta Adrenergic Blockade in eight dogs, after control measurements of hemodynamic parameters were obtained, a bolus injection of propranolol (Inderal; Ayerst Laboratories, New York, NY), 1 mg/kg was administered intravenously to block *beta* adrenergic receptors. *Beta* adrenergic blockade was verified by the absence of inotropic and depressor responses to a bolus injection of isoproterenol (Iprenol; Vitarine, New York, NY). Hemodynamic parameters were recorded and radioactive microspheres were injected to define a new control condition prior to nicotine infusion. Nicotine was then infused and a second injection of microspheres was made at peak reservoir volume.

Group3. After Combined Alpha and Beta Adrenergic Blockade in six dogs, after control measurements of hemodynamic parameters were obtained, *alpha* adrenergic blockade was produced with phenoxybenzamine HC1 (Dibenzyline; Smith Kline and French Laboratories, Philadelphia, PA), 2 mg/kg in 300 ml isotonic saline infused intravenously over 45 min. *Alpha* adrenergic blockade was verified by absence of press or response to a bolus injection of methoxamine (Vasoxyl; Burroughs Wellcome Co., Research Park, NC), 4 mg i.v. *Beta* adrenergic blockade was then produced and verified as described for Group 2. In dogs subjected to combined *alpha* and *beta* adrenergic blockade, arterial blood pressure was maintained near the pre-blockade control level by addition of blood from a donor dog. Radioactive microspheres were injected to define the pre- nicotine control condition subsequent to combined *alpha* and *beta* adrenergic blockade. Following this injection, nicotine was infused and at peak reservoir volume, hemodynamic parameters were recorded and a second injection of microspheres was made.

Group 4. After Combined Alph and Beta Adrenergic and Cholinergic Blockade in 4 dogs, after control measurements were obtained, *alpha* and *beta* adrenergic blockades were produced and verified as described above. Then cholinergic blockade was produced with atropine sulfate (Elkins-Sinn, Inc., Cherry Hill, NJ), 1 mg i.v.[19] This dose of atropine sulfate was adequate to

prevent the transient, vagally - mediated bradycardia which occurs initially up on intraveionous infusion of nicotine.

The result of George *et al*., 1983 showed that *Series I with Uncontrolled Aortic Pressure:* Under control conditions (before nicotine infusion) systemic hemodynamic parameters were unremarkable and Regional cerebral blood flow values showed regional heterogeneity (cerebral cortex; cerebellum > pons; medulla; spinal cord). Nicotine infusion increased mean aortic pressure (+72%), heart rate (+ 11%), and mean left atrial pressure (•+ 112%), but it had no significant effect on mean central venous pressure or aortic blood flow. Nicotine caused a significant increase in Regional cerebral blood flow throughout brain flow increased 67% in cerebral cortex, 38% in cerebellum, 46% in pons, 39% in medulla, and 48% in spinal cord. Regional blood flow is determined by the regional arteriovenous blood pressure gradient and by the regional vascular resistance [vascular resistance = (mean aortic pressure — mean central venous pressure)/ blood flow]. Since nicotine-induced increases in Regional cerebral blood flow values were accompanied by changes in the arteriovenous blood pressure gradient, vasoactive effects of nicotine in brain were assessed by comparing calculated values for regional vascular resistance. *Series II With Aortic Pressure Constant* With aortic pressure held constant without autonomic blockade (Group 1), nicotine reduced mean left atrial pressure (-50%) and mean aortic blood flow (-52%), but it had no other systemic hemodynamic effects. Under these constant pressure conditions, nicotine raised significantly flow in cerebral cortex (+38%), while it did not affect flow in any other region of brain. With pressure held constant after selective *beta* adrenergic blockade, nicotine caused significant reductions in mean left atrial pressure different percent, but it had no effect on Regional cerebral blood flow values. With pressure constant after combined *alpha* and *beta* adrenergic blockade (Group 3), nicotine caused a significant reduction in mean aortic blood flow, but it did not change other systemic hemodynamic parameters. Under these conditions, nicotine increased significantly Flow in cerebral cortex (+29%) while it had no significant effect on flow in any other region of brain. Additional cholinergic blockade (Group 4) did not cause further changes in either systemic hemodynamic parameters or Regional cerebral blood flow values during nicotine infusion.

Furthermore quantitative estimate on the *effect of nicotine on systemic hemodynamic parameter with uncontrolled aortic pressure and intact autonomic receptors* is shown in Table 1.

Table 1. The effect of nicotine on systemic hemodynamic parameter with uncontrolled aortic pressure and intact autonomic receptors (Source: George *et al*., 1983).

	Control	Nicotine
Mean aortic pressure (mmHg)	121.3±6.3	208.9±8.0*
Mean left arterial pressure (mmHg)	5.2±0.9	11±2.5*
Heart rate (beats/min)	148±7	165±9*
Mean central venous Pressure(mmHg)	3.8±0.8	4.7±0.9
Aortic blood flow (ml/min)	1216±178	1491±295

***:** *indicates significant difference at 0.05 levels. V*alues are expressed as mean ±SD

The Regional cerebral blood flow (rCBF) values showed regional heterogeneity (cerebral cortex; cerebellum > pons ; medulla; spinal cord) (Table 2).

Table 2. Regional heterogeneity of Regional cerebral blood flow (rCBF) values (Source**:** George *et al*., 1983**).**

	Control	Nicotine
Regional cerebral blood flow (ml/min per100g)		
Cerebral cortex	36±3	60±8*
Cerebllum	39±4	54±7*
Pons	28±3	41±5*
Medulla	28±3	39±5*
Spinal cord	21±2	31±4*
Arterial blood parameters		
Po_2 (mmHg)	117.6±6.2	114.6±5.4
Pco_2 (mmHg)	32.6±1.3	34.5±2.2
PH	7.36±0.01	7.34±0.01*
Hematocrit (%)	37.8±0.9	44.6±2.5*

Values are expressed as mean ±SD.

*: Indicates that results were significant compared to those the control group at P≤ 0.05 level.

Another study was conducted by Edward *et al*., (2000) to determine the effects of nicotine on regional cerebral blood flow in awake and resting human tobacco smokers. In this study nine male and nine female healthy adult smokers were used.

Subjects were male or female tobacco smokers who smoked 15–40 cigarettes per day, between 18–52 years of age, in good physical health, and not taking any medications (except oral contraceptives or replacement hormones). Individuals suffering from renal, hepatic, cardiovascular, hematological, neurological, psychiatric, or endocrinological disease were excluded. Fertile women who were not using an acceptable method of birth control (oral contraceptives, a barrier method, intrauterine device, or levonorgestrel implants) were excluded. Subjects were taught to use an FDA- (food drug administration) approved nicotine nasal spray device prior to the study day. Subjects reported to the PET suite, following overnight abstinence from tobacco products (.10 h). A sample of expired air was analyzed for carbon monoxide (CO) in parts per million (ppm). Expired air CO levels .10 ppm in the subject required an extensive interview to ascertain possible noncompliance with the no-smoking directive within the past10 h. Also, ECG and systematic arterial blood pressure were monitored throughout the study. Following an explanation of the imaging procedure, the subject lay supine in the PET gantry. For each subject, six PET scans were conducted at 12–15-min intervals using $H_2\ ^{15}O$ to determine Regional cerebral blood flow. The experimental session consisted of the following: scan #1—5% CO_2 in 95% O_2; scan #2—baseline; scan #3—nasal spray of oleoresin of pepper placebo; scan #4—recovery from pepper placebo; scan #5—nasal spray of nicotine; scan #6—partial recovery from nicotine. Both venous and arterial access lines were placed in the forearms of the subjects prior to the Regional cerebral blood flow sessions for obtaining blood for plasma nicotine concentrations and arterial p_{CO2} and p_{O2} levels. About 3 min before the third and fifth scans, either placebo or nicotine spray (0.5 mg/ spray) was administered intranasally. A total of 2–5 sprays were administered, depending on the volunteer's ability to tolerate the nicotine. Arterial and venous blood samples for nicotine and cotinine were withdrawn before and after the fifth and sixth scans. Samples were drawn prior to, and about 3, 6, 10, 15, 20, and 30 min following nicotine administration. Blood samples were collected in standard 5 ml EDTA vacutainer tubes and stored immediately on crushed ice. Immediately following completion of the study the samples were centrifuged and plasma aliquots were frozen at -20°C until analysis. Venous and arterial samples were analyzed for nicotine and cotinine using HPLC techniques.

The result of the study on plasma nicotine by Edward *et al*., (2000) revealed that nicotine had cardiovascular effects. The nicotine plasma levels decreased rapidly, consistent with its known alpha $t_{1/2}$ of about 8 min. Before nasal pepper, the mean heart rate ± SD was 61.956±10.04/min and after 65.69 6± 9.06/min ($P > 0.05$). This slight increase continued for at least 15 min. After nasal nicotine, the mean heart rate ± SD was 92.74 6± 11.33/min ($P < 0.001$). The nicotine-induced heart rate increase returned gradually toward control levels over the next 30 min, but was still elevated. The mean± SD peak systolic arterial blood pressure before nasal pepper placebo was 116.22 6± 10.76 mm Hg and after120.72± 11.57 mm Hg ($P < 0.05$). The mean ± SD systolic blood pressure after nasal nicotine spray was 132.63 6 ±11.94 mm Hg ($P < 0.001$). The mean ± SD diastolic blood pressure before nasal pepper spray was 68.21 6 ±7.54 mm Hg and after 72.17 6 ±9.52 mm Hg ($P > 0.05$). The mean ± SD diastolic blood pressure after nasal nicotine spray was 80.16 6 ±9.49 mm Hg ($P < 0.001$).

Nicotine nasal spray increased normalized cerebral blood flow in multiple structures of the brain. A large increase in cerebral blood flow was seen in the cerebellar hemisphere and vermis. Increased blood flow was also noted in the pons and thalamus bilaterally in these deep brain structures. Nicotine induced a neocortical increase in cerebral blood flow in the pre motor cortex bilaterally, in the primary visual cortex, peaking in the lingual gyrus, and right inferior temporal gyrus. There was a significant increase in blood flow within the rectal gyrus. However, a large increase in blood flow in the nasal cavity (presumably due to a direct mucosal effect) partially obscured the increase in the rectal gyrus. The active pepper placebo spray produced an overlapping, but also distinct pattern of normalized increased brain blood flow compared to that of nicotine spray. The overlapping areas of increased flow included the cerebellar hemisphere, vermis, premotor cortex, and in rectal gyrus. An increase in cerebral blood flow in the right ventral anterior cingulated cortex and left anterior insula was unique to the pepper placebo. The overlapping areas of increased blood flow presumably represent brain structures responding to nasal irritation caused by both the nicotine and pepper sprays. The mean normalized cerebral blood flow effects of nicotine spray minus those of the pepper spray were performed to determine more specifically the effects of nicotine not due to nasal irritation. The correlation analysis between arterial nicotine levels and changes in normalized regional brain activities

showed marginally significant “negative” correlation in the left parietal operculum (*Z* 5 24.3) and in the region of the left hippocampus/parahippocampa gyrus (*Z* 5 24.2,) (Figure 2 and 3).

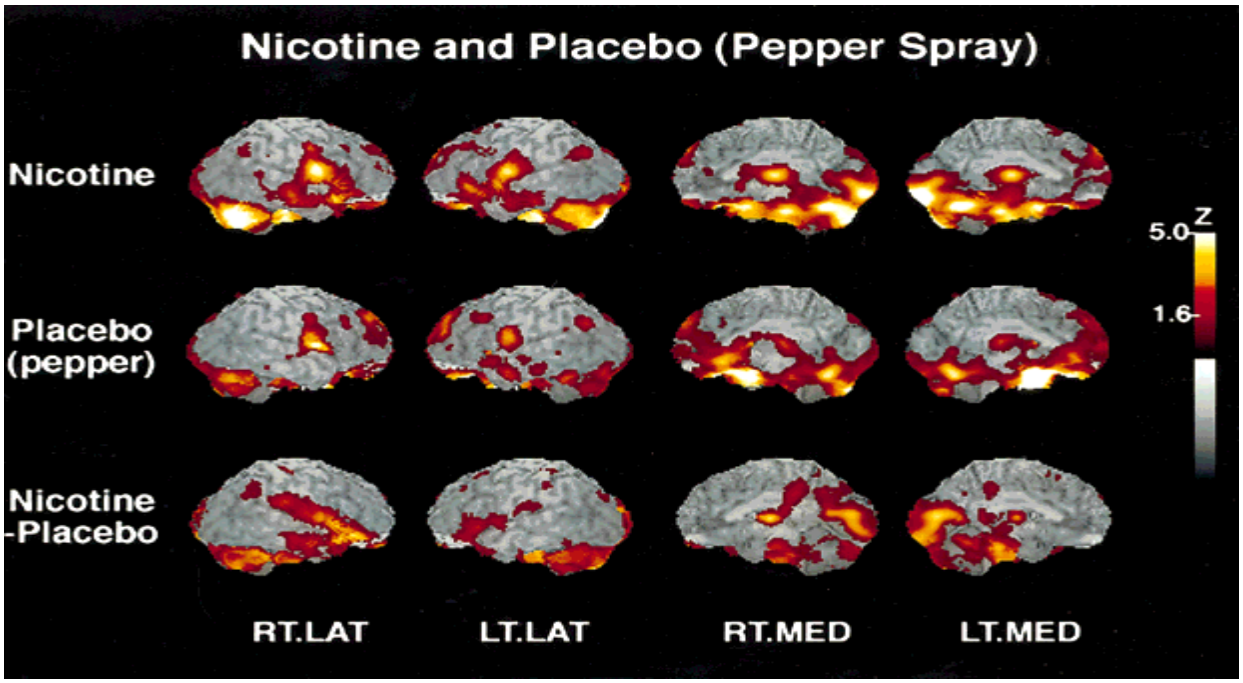

Figure 2. Nicotine and Regional cerebral blood flow (rCBF) (Source: Edward *et al*., 2000)

Correlation between regional blood flow and arterial nicotine levels was shown with Figure 3. A negative correlation indicates that Regional cerebral blood flow decreases relative to an increase in arterial nicotine concentration. A positive correlation indicates that Regional cerebral blood flow increases with an increase in arterial nicotine concentration. The brain slice levels in stereotactic coordinate.

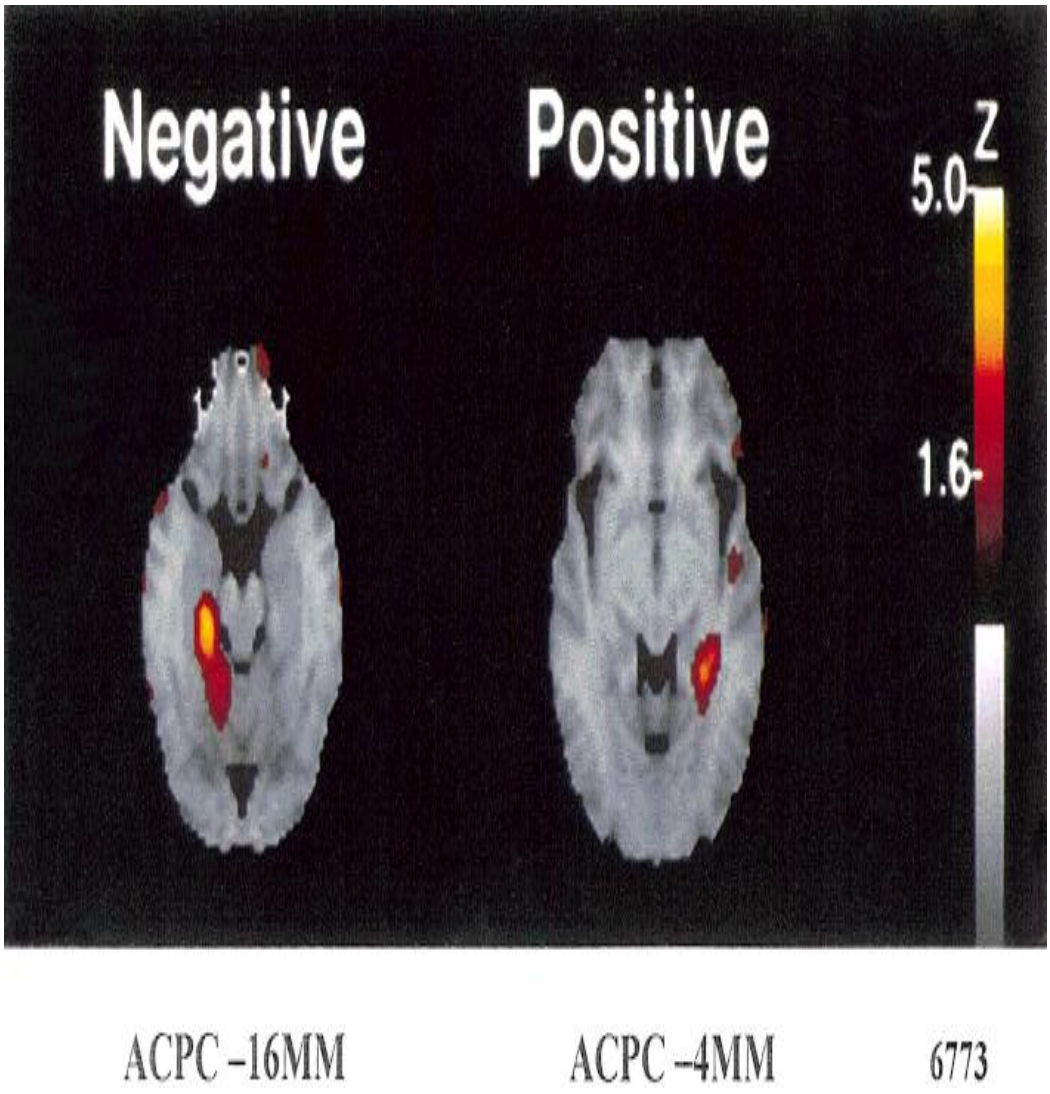

Figure 3. Negative and positive correlation between regional blood flow and arterial nicotine (Source: Edward *et al*., 2000).

Parahippocampal gyrus demonstrated a trend of "positive" correlation (Z 5 3.4, Figure 3). The analysis of gender differences did not show any brain structures where brain responses to nicotine were statistically different between male and female subjects. The results of 5% CO_2/95% O_2 inhalation demonstrated three foci of significant relative regional increase in brain activities (Figure 2). Except for the activation in the gyrus, there was no overlap between changes seen with nicotine spray and those with CO_2 inhalation. The mean ± arterial $_{p}$**CO2** for scan 1 was 38.3 6 ±4.5 mm Hg immediately after inhalation of 5% CO_2/95% $\mathbf{O_2}$ and 35.8 6± 3.7 mm Hg after approximately 12–15 min breathing room air for scan 2. The mean ± SD increment in pCO_2 was 2.4 6 ±2.9 mm Hg.

In contrast the mean ± SD pO2 for scan 1 was 271.9 6± 63.4 and for scan 2 89.4 6± 14 mm Hg. The mean ± SD increment in pO2 was 182.5 6 ±64.8. Therefore, the pO2 increased, where as the pCO2 only increased negligibly during 5% CO_2/95% O_2 inhalation. In these tobacco-smoking volunteers who were abstinent overnight and breathing only room air, the mean pCO2 and pO2 levels did not change. They were similar in the normal range for the remaining five PET scans.

In a study conducted by Aytu¨lO¨nla *et al*., 2003 on Alterations of brain tissue in fetal rats exposed to nicotine in utero were investigated. In addition, possible involvement of nitric oxide and catecholamines in the nicotine induced abnormalities of developing brain, behavioral and histopatological alternations were studied. In this study female wrist rats weighing 180–200 g were used. Rats were randomized and assigned to four groups: one control and three treated groups.

These rats were housed in plastic cages in groups of five without any contact with the male rats until mating under a standard 12/12-h light/dark cycle in a room maintained at 22 ± 3^0C. Mating was performed between 5:00 p.m. and 8:30 a.m. by the addition of a male rat into each cage. Gestational day (GD) 1 was assumed to start 24 h after onset of the mating period. Pregnant rats were divided into four experimental groups. Starting from GD1, Group1 received 0.2ml/day saline (0.9% NaCl) subcutaneously; Groups 1, 2, and 3 mg/kg/day nicotine hydrogen tartrate (NHT) in 0.2 ml saline sc, respectively, for 20 days (corresponding to 0.35, 0.7, and 1.05 mg/kg/day base nicotine, respectively). The injections were performed at 5:00 p.m. every day and the last nicotine injection was at 5:00 p.m. of GD20. Blood was withdrawn by intra cardiac puncture under ether anesthesia next morning and fetuses were removed by laparotomy. Sizes of fetuses from the same mother, fetal body weights, and placental weights were recorded. Brains were dissected, freed off blood by washing with saline, and some of the brains were frozen at -80 ^{0}C for further analysis of nitrate + nitrite and monoamine levels, which were measured in the whole fetal brain. Rest of the fetal brains were fixed in 10% formalin for 48 h for the morph metric analyses with light microscopy and fixed in 2.5% phosphate-buffered glutaraldehyde (pH 7.4) at 4 ^{0}C overnight for electron microscopic evaluation.

For determination of nitrite + nitrate levels Defrosted brain tissue samples were homogenized in phosphate buffer (pH 7.5; 1/5, w/v) and centrifuged at 2000xg for 15 min. Supernatant (0.1 ml)

was added to 0.9 ml distilled water, 0.25 ml of 0.3 M NaOH, and 0.25 ml of 5% (w/v) ZnSO4 for deproteinization. This mixture was then centrifuged at 2000xg for 15 min and the supernates were used for the assay. Nitrate levels in the whole brain supernates were determined spectrophotometrically based on the reduction of nitrate to nitrite by nitrate reductase (EC 1.6.6.2) from Aspergillus species, in the presence of NADPH and FAD (Taskiran *et al*., 1997). Nitrite levels were then measured by Griess reaction (Green *et al*., 1982). Sodium nitrate dilutions were used for standard measurements. Nitrite + nitrate levels were normalized to wet weight of the tissue used.

For determination of catecholamine levels defrosted brain tissue samples were homogenized in homogenization solution (1/5, w/v) composed of 0.025% cysteine and EDTA in 0.15 M hydrochloric acid and centrifuged at 12,000 xg for 10 min at 4^0C. Norepinephrine,epinephrine,dopamine,3-methoxy-4 hydroxyphenylethyleneglycole (MHPG), normetanephrine (NMN), homovanilic acid (HVA), and 3,4-dihydroxyphenylacetic acid (DOPAC) levels were analyzed in the whole brain supernatants by high-performance liquid chromatography (LC-6A Shimadzu) with electrochemical detection. Briefly, 50 Al of the supernatants were dissolved in the mobile phase consisted of an aqueous component and acetonitrile (181/ 19, v/v; aqueous component in mM: citric acid 12.16, (NH4)2PO4 11.60, sodium octylsulfonate 2.54, dibutylamine phosphate 3.32, disodium EDTA 1.11, pH 3.8), filtered through a 0.45-Am filter, and degassed. The sample was injected into the system with a flow rate of 1 ml/min under approximately 170 bars of pump pressure. Norepinephrine, epinephrine, dopamine, MHPG, NMN, and DOPAC dissolved in 0.2 M hydrochloric acid/0.025% cysteine and EDTA (1/9, v/v), and HVA dissolved in ethanol/0.025% cysteine and EDTA (1/9, v/v) were used as the internal standards. Monoamine levels were normalized to wet weight of the tissue used.

The results of Aytu¨lO¨nala, *et al*., (2003) experiments are presented as follows:

Effects of nicotine on fetal, fetal brain, and placental weights

Nicotine exposure inhibited fetal weight [$F(3,212) = 7.84$, $P < 0.001$] and fetal brain weight [$F(3,157) = 5.65$, $P < 0.01$] significantly at all doses, however, placental weights remained unchanged [$F(3,206) = 1.19$].

Effects of nicotine on nitrite + nitrate levels in fetal brain tissue

In utero exposure to 1 mg/kg nicotine did not affect nitrite + nitrate levels in the fetal brain tissue, however, nitrite + nitrate levels in the brains of fetuses exposed to 2 and 3 mg/kg nicotine during intrauterine life were significantly lower than the control and 1 mg/kg nicotine group [$F(3,64) = 8.50$, $P < 0.001$].

Effects of nicotine on monoamine levels in fetal brain tissue

In utero exposure to 2 and 3 mg/kg nicotine significantly increased norepinephrine and NMN levels in the fetal brain tissue [$F(3,35) = 26.40$, $P < 0.0001$, and $F(3,37) = 5.41$, $P < 0.01$, respectively], while only exposure to 3 mg/kg nicotine significantly increased dopamine, MHPG, and DOPAC levels [$F(3,53) = 3.30$, $P < 0.05$;$F(3,43) = 3.30$, $P < 0.05$, and $F(3,41) = 4.57$, $P < 0.01$, respectively]. Epinephrine, and HVA levels in the fetal brain tissue remained unchanged after in utero exposure to nicotine.

Effects of nicotine on fetal brain morphology

Morphometric analyses on the light microscopy showed significant decreases in the number of cells in dorsal hippocampus CA1 area of the fetal brains in utero exposed to 1, 2, and 3 mg/kg nicotine [$F(3,15) = 6.95$, $P < 0.01$]. Electron microscopic evaluation of nicotine- induced alterations in dorsal hippocampus CA1 area showed the photomicrograph of dorsal hippocampus CA1 area in the control group. Electron microscopy defined normal pyramidal neuronal morphology showed euchromatic nucleus and regular nuclear membrane without any chromatin condensations or disruptions in the nucleus. Cytoplasmic examination revealed rough endoplasmic reticulum dense in the pericarion, normal mitochondrial morphology. In utero exposure to nicotine resulted in a dose dependent intracytoplasmic edema formation, mitochondrial swelling, dilation of rough endoplasmic reticulum, and ribosomal accumulations as well as nuclear configurative abnormalities and membrane disruptions and also condensation of the nuclear chromatin.

Furthermore, quantitative estimate on the effect of nicotine on fetal, fetal brain, placental weights and mean number of cells per area of brain dorsal hippocampus CA1 region from control fetal rats and from fetal rats exposed to NHT for 20 days during intrauterine life are shown in Table 3 and Table 4 below.

Table 3. Mean placental weights and body and brain weights of fetuses from control Dams and from dams exposed to NHT for 20 days during pregnancy (n = 25– 85**)** (Source: Aytu¨lO¨nala *et al*., 2003).

	Fetal body Weight(g)	Fetal brain Weight(mg)	Placental Weight(mg)
Control NHT(mg/kg/day)	3.54±0.18	156±7.2**	450±9.5
1 mg/kg/day	2.98±0.12*	132±4.2**	430±7.5
2 mg/kg/day	2.51±0.11***+	131±4.2**	450±8.5
3 mg/kg/day	2.49±0.23***+	122±7.9**	460±10

**: Indicate that results were significantly different compared to controls at P≤ 0.01.

*****+**: indicated significant difference at P≤ 0.05 level.

Table 4. Morphometric analysis showing mean number of cells per area of brain dorsal hippocampus CA1 region from control fetal rats and from fetal rats exposed to NHT for 20 days during intrauterine life (n=5) (Source: Aytu¨lO¨nala *et al*., 2003).

	Cell number per area
ControlNHT(mg/Kg/day)	25.70±1.34
1 mg/Kg/day	20.94±1.30*
2 mg/Kg/day	19.35±1.58*
3 mg/Kg/day	18.80±0.40***

*******: indicated significant difference at P≤ 0.05 level.

Machaalani *et al.,* 2006 conducted a study on the effect of nicotine in apoptotic marker of seven nuclei of brain steam, caudal medulla and two sub- region of hippocampus. They used twenty eight postnatal male and female piglets. The piglets were randomized and assigned in two groups: control and treated groups..

Mixed-breed miniature piglets were transported from a commercial piggery and underwent aseptic surgery for i.p. insertion of an osmotic mini pump (Alzet; Alza Corporation, USA, Model 2ML2), under general anesthesia within 2 days of birth. Inhalational anesthesia was induced using a facemask delivering 1–3% isoflurane with 30–50% nitrous oxide in oxygen, and

maintained throughout surgery with adjustment according to the level of spontaneous respiratory efforts and heart rate. Analgesia was delivered after induction of anesthetic, and prior to surgery, with paracetamol via rectal suppository (100 mg/kg). Antibiotic therapy (cephalexin; 10 mg/kg) commenced intra-operatively and continued for 2 days. Piglets in the control group were implanted with a mini pump containing sterile water only. Whereas Piglets in the treatment group received nicotine hydrogen tartrate salt (Sigma-Aldrich, Germany; N5260) dissolved in sterile water (Water for injections BP; Astra, Australia) at a rate of 2 mg/ kg/day for 2 weeks. This dose was chosen after a pilot study of six piglets receiving a dose of 1.44 mg/kg/day showed that four piglets had no measurable cotinine in the serum at the end of the study period, and at a dose of 2.9 mg/kg/day, two of three piglets died suddenly, 1 and 2 days after implantation of the capsule. After implantation of the capsule, piglets were housed in groups in an animal facility, but nicotine-treated piglets were always caged separately to control piglets to prevent cross-contamination of nicotine through urine and/or other body fluids. On day 13–14 all animals were killed painlessly with an overdose of pentobarbitone. Blood and urine samples were collected for analysis of cotinine levels and the brain was removed for histological evaluation. A 10 ml sample of cardiac blood was collected after killing, for measurement of cotinine levels. The blood was extracted, placed into a sterile, plain tube, and left to clot on ice for 1 h. Serum was then separated by centrifuge. Cotinine levels in the serum and urine samples were measured in the Biochemistry Department at Royal Prince Alfred Hospital, Camperdown, New South Wales, Australia, using gas chromatography mass spectrometry with selected ion monitoring. To mimic the preservation techniques used for SIDS infants, the whole brain, down to the spino-medullary junction was removed fresh, weighed, and then stored in 10% formalin for 14 days. The brainstem and hippocampus were then detached and cut into 4 mm slices, placed in cassettes and returned to the formalin solution for a further 5 days. Cassettes were then washed in 70% ethanol (6 h), processed over 3 days, and tissue slices paraffin embedded. Transverse 7-m sections at the level of the brainstem caudal medulla, and from the hippocampus, were cut, mounted on slides coated with 3-aminopropyltriethoxysilane, and stored in dust free conditions at room temperature. The results of Machaalani *et al*., 2006 showed as follows by comparing control and treated group.

Piglet characteristics: A total of 28 piglets were studied, and piglet characteristics are shown. There was no statistically significant difference among groups for any of the parameters

measured including, age, body weight at surgery and at death, average daily weight gain, or brain weight.

Cotinine concentrations: For the nicotine-exposed animals, the mean serum and urine concentrations of cotinine measured at the end of the study were 20.3_2.5 ng/ml (males: 19.8_5.0; females: 20.8+_1.1), and 92.7_21.8 ng/ml (males: 74.6_35.6; females: 113.7_ 20.1), respectively. Although urinary concentrations tended to be higher in females, there was no statistically significant difference between genders for cotinine concentrations.

Active caspase-3: Among the control groups (females vs males), females had less active caspase-3 in the DMNV and Cun compared with males (*P*_0.02, and *P*_0.05, respectively; No differences were found between the genders in the hippocampus. The finding that at baseline, males have a greater number of active caspase-3 neurons than females in the DMNV and Cun, suggests that there is a baseline difference in neuronal turnover, between genders for the DMNV and Cun. Among the nicotine-exposed groups (females vs males), females had significantly less active caspase-3 in the Grac (*P*_0.006) compared with males No differences were found between the genders in the hippocampus. Thus, the baseline difference between genders in the DMNV and Cun no longer exists after nicotine exposure. In the Grac, males had greater active caspase-3, suggesting a greater vulnerability to the effects of nicotine, of neurons in the male compared with the female Grac. Comparing nicotine-exposed to control piglets, changes in active caspase-3 were gender and region specific. In general, piglets with nicotine exposure had more active caspase -3 than controls. Compared with control males, nicotine-exposed males had more positive staining for caspase-3 in the XII (*P*_0.01) and Grac, and in the dentate gyrus. Compared with control females, nicotine-exposed females had more caspase-3 in the DMNV, XII and, and NSTT. Thus, the XII is affected by nicotine exposure regardless of gender, while the Grac and dentate are affected in males, and the DMNV and NSTT are affected in females.

Teminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL)

Among the control group, females had less DNA fragmentation in the DMNV (*P*_0.04) and NSTT compared with males. Thus, at baseline, males have greater DNA fragmentation positive neurons than females in the DMNV and NSTT, suggesting higher rates of neuronal turnover in the DMNV and NSTT of males compared with females. Among the nicotine-exposed groups, females had fewer DNA fragmentation positive neurons in the NSTT (*P*_0.02), with non-significant trends to lower expression in all other nuclei of the medulla compared with males. No

gender effect was observed in the hippocampus. The finding of fewer DNA fragmentation positive neurons in the NSTT of females compared with males, even after nicotine exposure, suggests that nicotine had no effect on the NSTT; however, it did on the DMNV since the baseline difference in genders is no longer present. Comparing nicotine-exposed to control piglets, no differences were observed for DNA fragmentation between the groups, for any of the brain regions analyzed although a trend to increase was observed in the Cun of the nicotine exposed compared with control males.

Furthermore quantitative estimate shows the characteristic data for the control and nicotine-exposed piglet groups (average SEM) (Table 5).

Table 5. Characteristic data for the control and nicotine - exposed piglet groups (average ±SEM) (Source: Machaalani *et al*., 2006)

	Control (male)	Control (female)	Nicotine (male)	Nicotine (female)	P value
Number of piglets	7	7	7	7	_
Age at pump implant (days)	0 - 2	0 – 2	0 - 2	0 - 2	_
Age at death (days)	13 - 14	13 – 14	13 - 14	13 - 14	_
Body weight at implant (kg)	1.04±0.09	1.40±0.10	1.27±0.09	1.12±0.09	0.06
Average daily weight gain	71.1±21.8	58.9±8.6	59.7±13.1	67.4±8.3	0.91
Body weight at death (kg)	1.88±0.33	2.07±0.10	2.00±0.22	1.93±0.11	0.96
Brain weight (g)	37.8±0.90	37.1±0.48	36.7±1.07	38.1±0.75	0.62
Brain: body weight ratio (%)	2.4±0.4	1.8±0.5	2.0±0.2	2.0±0.1	0.5
Serum cotinine (ng/ml)	0.0±0.0	0.0±0.0	19.8±5.0	20.8±1.1	-.001
Urine continine (ng/ml)	0.0±0.0	0.0±0.0	74.6±35.6	113.7±20.10	0.002

The values are expressed as mean ± SEM.

A comparison between genders in the control and nicotine-exposed piglet groups is shown in Table 6.

Table 6. Comparison between genders in the control and nicotine-exposed piglet groups on status of brain nuclei **(**Source**:** Machaalani *et al*., 2006**).**

Nucleus	control	control	-	nicotine	nicotine	-
Medulla	Male	Female	P-value	Male	Female	P- value
XII	14.6±3.8	18.0±4.0	0.57	43.3±8.3	37.6±5.8	0.59
DMNV	20.2±3.5	8.6±1.4	0.02	18.4±5.4	23.5±4.2	0.47
NTS	10.8±2.9	5.0±0.9	0.17	9.8±2.4	10.1±2.7	0.95
Grac	15.8±3.1	8.9±2.0	0.10	28.4±4.1	10.8±3.4	0.006
Cun	16.3±2.5	9.5±1.5	0.05	22.5±10.4	12.9±4.3	0.42
NSTT	15.2±3.4	7.8±1.3	0.11	28.1±7.2	19.5±4.9	0.35
ION	23.3±7.3	9.1±3.5	0.16	25.3±8.3	7.0±1.9	0.05
Hippocampaus						
Dentate	12.5±4.6	24.6±5.2	0.07	29.7±4.1	23.4±2.9	0.24
CA4	23.0±6.6	25.6±9.1	0.81	22.2±5.1	36.3±7.7	0.16

More active caspase -3 positive neurons were observed in the XII, DMNV and Grac of nicotine-exposed piglets compared with controls. Scale bar - 80 - m (Figure 4).

- Results are expressed as mean ± SD.

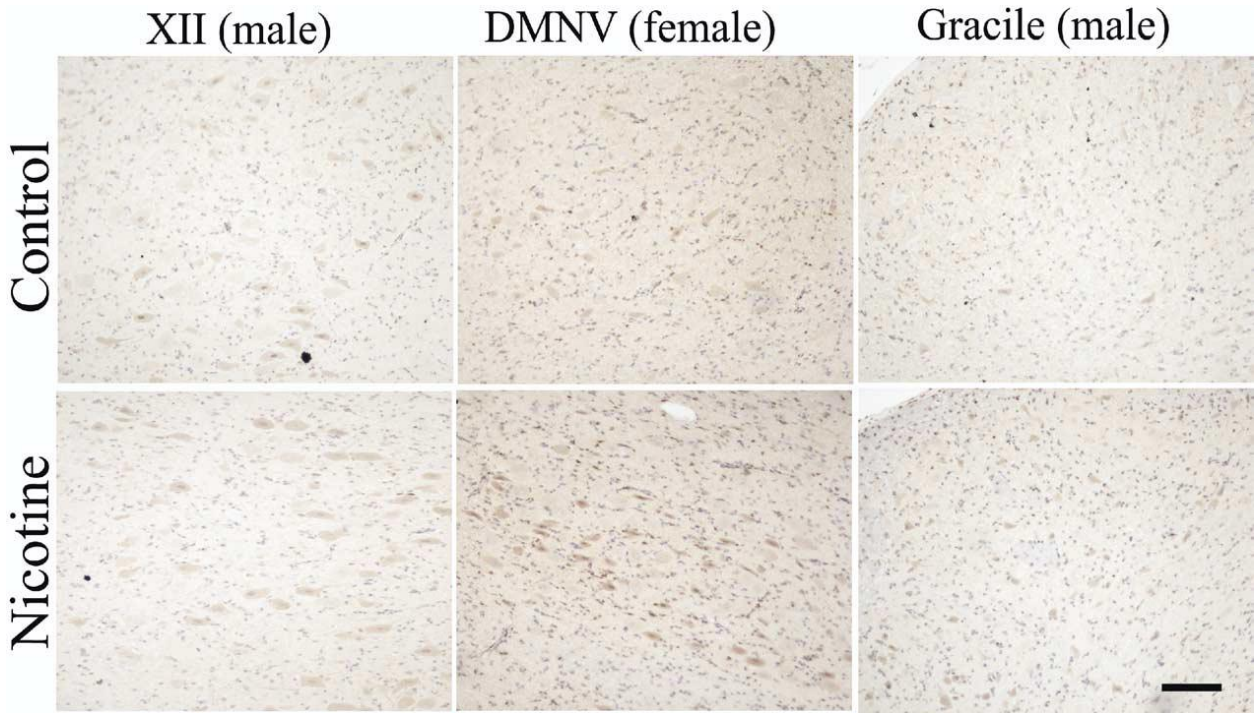

Figure 4. Micrographs illustrating active caspase-3 staining in control piglets (top panel) vs nicotine-exposed piglets (bottom panel) (Source: Machaalani *et al*., 2006).

Omotoso *et al*., 2013 investigated morphological and neurohistological changes observed in the cerebral cortex of young Wistar rats exposed to nicotine during gestation. In this study twenty-four adult female Wistar rats (*Rattus norvegicus*) with mean weight 215.83 ± 6.71 g were used. The rats were housed in wire-gauzed in with ages good ventilation, feeds and water were provided for the rats throughout the period of the experiment. Vaginal smearing was done as earlier performed and the oestrous phase of each female Wistar rat was determined. The rats were grouped according to their oestrous phases, and male rats were introduced to the female rats, overnight, in their proestrous phase. Pregnancy was confirmed through vaginal smears early hours of the following morning after perceived mating, as indicated by the presence of mucous plug and sperm cells in the vaginal smear.

The rats were divided into 3 main groups (Group A - first trimester, Group B - second trimester and Group C - third trimester) with each group subdivided into 2 subgroups (1 and 2). The 3 major groups represented rats in the 3 trimesters of gestation period (each being a duration of 7 days, with a total of 21/22 days in Wistar rats) while the subgroups 1 and 2 represented the control and treated groups respectively. Treated animals received 0.06 mg/kg nicotine in 0.1 ml of vehicle in two divided doses, while the control animals received 0.1 ml normal saline intra-

peritoneally, for six (6) consecutive days within each trimester. The pregnant animals were allowed to litter, and at postnatal day (PND) 35, the pups were sacrificed by cervical dislocation. The skull of each pup was dissected to expose and remove the brain. The brain was weighed and the parietal and temporal cortices excised, and fixed in 4% par formaldehyde to prevent autolysis and putrefaction. The fixed tissues were processed for histological examination using the cresyl fast violet staining technique, to demonstrate Nissl bodies in the neuronal cytoplasm and the general architecture of the parietal and temporal cortices, of both the control and nicotine-exposed offspring.

A result of Omotoso *et al*., 2013 showed that the weights of the offspring recorded at birth showed reduction in the birth weight of pups exposed to nicotine during the 1st and 2nd trimesters, compared with their respective Control; and this was significant ($p<0.05$) in the 2nd trimester group. The birth weight of pups exposed to nicotine in the 3rd trimester was not taken. Weight of animals on postnatal day 35 increased in both the 2nd and 3rd trimester treated groups, compared with their respective controls. Although this weight change was not statistically significant in the 2nd trimester group ($p>0.05$), it was however significant in the 3rd trimester group ($p<0.05$). The body weight of nicotine-exposed rats in the 1st trimester significantly reduced when compared with the control group ($p<0.05$). Brain weight revealed a significant decrease in the 1st trimester group ($p<0.05$), a slight increase in the 2nd trimester ($p>0.05$), and a non-statistically significant reduction in the 3rd trimester ($p>0.05$). The brain weight difference between the nicotine-exposed groups of the 2nd and 3rd trimesters was significant ($p<0.05$).

Furthermore photo micrographic estimate of parietal cortices show variation among control and treated groups of rats (Figure 5).

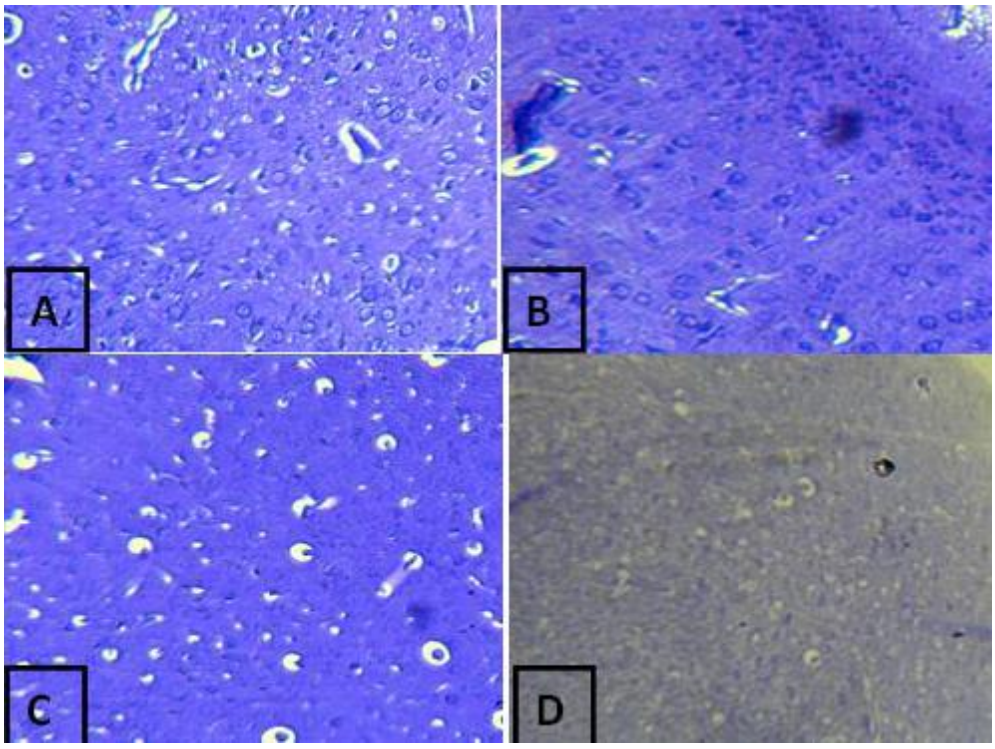

Figure 5. Photomicrographs of the parietal cortices of control rats (A) and those exposed to nicotine in the 1st (B), 2nd (C) and 3rd (D) trimesters, showing slight increase in Nissl staining in B and C, but reduced in D. Vacuolations were more in the treated groups especially in C (Source: Omotoso *et al*., 2013).

The other photomicrographs estimate of temporal cortices show control rats (A) and those exposed to nicotine in the 1st (B), 2nd (C) and 3rd (D) trimesters, with increased vacuolar spaces in nicotine-exposed groups. The histological sections of the parietal and temporal cortices of the treated groups were slightly more intensely stained than the Control, especially those exposed to nicotine in the 1st and 2nd trimesters, reflecting increased positivity for m Nissl bodies (Figures 1 and 2). Variation was noticed in the 3 superficial layers of the cortex that were visible in the sections between the treated and control groups. Presence of vacuolar spaces was more in the parietal and temporal cortices of rats exposed to nicotine, especially in the 2nd and 3rd trimester, when compared with the control (Figure 6**).**

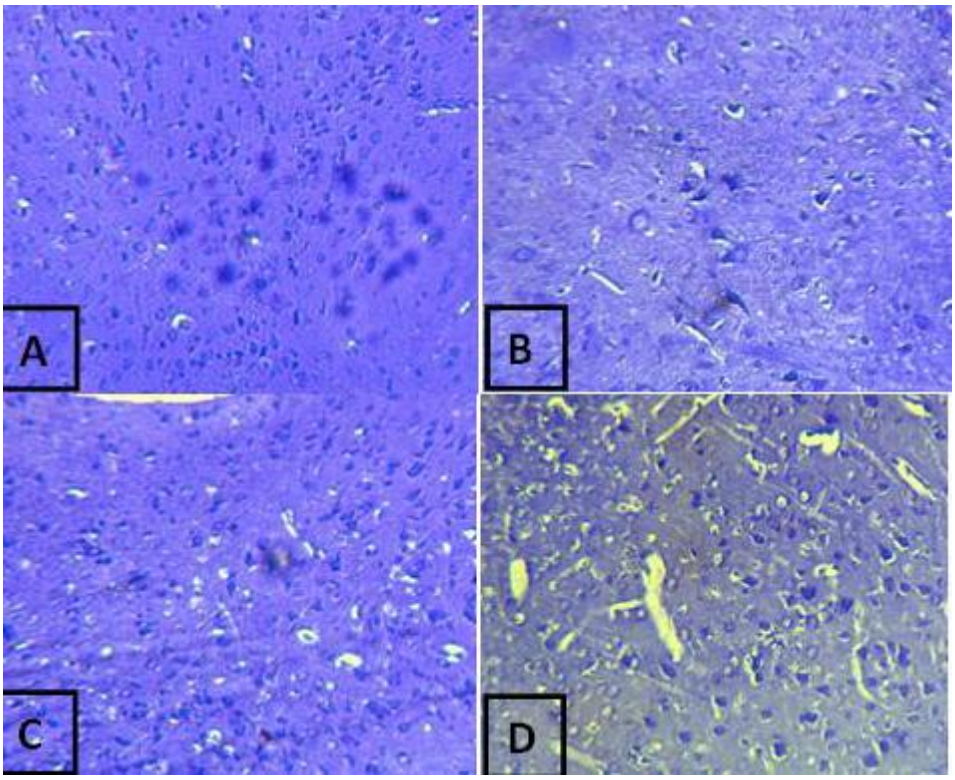

Figure 6. Photomicrographs of the temporal cortices of control rats (A) and those exposed to nicotine in the 1st (B), 2nd (C) and 3rd (D) trimesters, with increased vacuolar spaces in nicotine-exposed groups. CFV (x100) (Source: Omotoso *et al*., 2013).

3. Discussion

Scientific literature reviewed in the present paper showed that exposure to nicotine produced morphological changes of the brain, neurotoxicity, histopatological change, destruction of motor and cerebral cortex activities.

The study conducted by George *et al*., (1983) has revealed histopatological effect of nicotine on regional blood flow of cerebral cortex, cerebellum, pons, medulla and spinal cord. This was evidenced by a definite change in regional cerebral blood flow (rCBF) through the brain, mean aorti pressure, heart rate and mean left atrial pressure of experimental groups, but the effect showed heterogeneity in different part of the brain. This change in the dogs appeared to be directly proportional to the strength of nicotine administered through intravenous infusion and the aortic pressure at which nicotine were infused. Increase in blood flow in all regions of the brain was observed during intravenous infusion in dogs with uncontrolled aortic pressure. However, with the exception of cerebral cortex, these increases in flow were less than proportional to the increase in aortic pressure. With pressure controlled, nicotine had no effect on blood flow in any region of brain, except cerebral cortex, where hyperactive response was seen.

The results of the study conducted by George *et al*., (1983) are comparable in few aspects with the results of the study conducted by Edward *et al*., (2000) on human subjects. In this study, in addition to brain damage nicotine also resulted in abnormality in regional cerebral blood flow, cerebllar hemisphere, vermis, cardiovascular system, plasma arterial concentration, plasma venous concentration, heart rate, systolic arterial blood pressure, and diastolic blood pressure. In study conducted by George *et al*., (1983) radioactive microsphere was used to produce a clear image on regional cerebral blood flow, whereas the study by Edward *et al*., (2000) used PET imaging machine to produce a clear image on the effect of nicotine in regional cerebral blood flow. The PET data from the mean postplacebo scan #3 was subtracted from the mean post nicotine scan #5 to show clear image that, nicotine increased regional cerebral blood flow in the thalamus, pons, and area 17 of the visual cortex. In addition to the above observations the study by Edward *et al*., (2000) showed that abstinence from tobacco products for some time increase the sensitivity to the odor of nicotine.

In another investigation Aytu¨l O¨nal *et al*., (2003) reported histopatological change and abnormalities in the fetal weight, fetal brain weight, monoamine level in fetal brain tissue, fetal brain morphology and decreased number of cells in hippocampal CA1 area in rats treated with nicotine. In addition to these effects nicotine caused disruption of nuclear membrane, formation of irregular nuclear, condensed nuclear chromatin, significantly increase cerebral arteries resistance and reduced total brain weight. The intrauterine exposure of fetal brain to increased concentration of nicotine also resulted in decrease level of nitric oxide metabolites in all region of the fetal brain. Apart from its indirect effects through reducing cerebral blood flow, nicotine has been reported to be a direct neuroteratogen in rat even at very low concentrations, specifically targeting brain development. The effects and extent of damage caused by nicotine in different parts and metabolite levels were dose-dependent.

The finding of Machaalani *et al*., (2006) also revealed histopatological changes in different parts of the brain and brain systems specifically in apoptotic marker in piglets. The distribution of apoptotic marker in piglet brain steam and hippocampus region was influenced by gender and nicotine exposure during early postnatal development. However, the gender difference was found in control group of animals without nicotine exposure. Gender appeared to determine the regional distribution of brain cell weakness to change in apoptotic expression. The apoptotic markers in this study indicated that nicotine induced more changes in active caspase-3 than DNA fragmentation. This effect could be related to the duration of nicotine exposure, the time of study, the concentration of nicotine, and the brain regions. Body weight changes were reported in four of the five studies reviewed in this project paper. Finally, result of this study gave extra information from the above studies of nicotine exposure. This might be due to difference in experimental animal species, duration of the exposure, dose of nicotine and gender.

In a study conducted by Omotoso *et al*., (2013) various morphological and neurohistological changes in different parts of the brain including the cerebral cortex were observed. Effects were seen from the first trimester of pregnancy to the third trimester of pregnancy particularly on parietal and temporal cortices in an in increased manner. In addition, the exposure in the trimesters caused spontaneous abortion, premature delivery and cytoarchtecture abnormalities in

the temporal and parietal lobes of cerebral cortex of treated group compared with control group. Damage of brain nuclei were reported in two of the five studies reviewed in this project paper.

This was good evidence that nicotine caused problems in hearing, short term memory and other several health problems. Additionally, nicotine administered either by smoking or by smokeless routes is considered the major appetite-suppressing component of tobacco, and since it is the major addictive component. It reduces appetite and alters feeding patterns typically resulting in reduced body weight.

4. Conclusion

Most of the research articles reviewed in the present project paper indicated that, the infusion of nicotine affects the functional and histological system of cerebral cortex and different parts of the brain. These effects of nicotine were demonstrated in different species of animal models; adolescent and fetal rats, postnatal piglets, human and dogs subjects with different doses of nicotine, exposure of time and exposing mechanisms. In addition, the presence of any dose of nicotine in the brain caused morphological disorder and other changes, including heart rate, plasma arterial concentration, plasma venous concentration, mean atrial pressure, mean aortic pressure, and histopatological alternation in the brain. All the study reviewed showed different toxicological effects of nicotine with respect to the animal model, doses used, duration of the experiment, infusion mechanism, and methodology used.

Therefore, nicotine caused a wide damage in different parts of the brain and neurological systems.

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