Flash visual evoked potentials and early visual development in infants born to drug misusing mothers

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No part of this thesis has been submitted in support of an application for another degree or qualification of this or any other university.

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List of Contents

List of Tables

List of Figures

List of publications based on thesis

Hamilton R, **McGlone L**, MacKinnon JR, Russell HC, Bradnam MS, Mactier H. Ophthalmic, clinical and visual electrophysiological findings in children born to mothers prescribed substitute methadone in pregnancy. Br J Ophthalmol 2010 Jun; 94 (6): 696-700.

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Abbreviations

IUGR	Intra uterine growth restriction
NAS	Neonatal abstinence syndrome
FAEE	Fatty acid ethyl ester
DVM	Delayed visual maturation
CVI	Cerebral visual impairment
VEP	Visual evoked potential
SNR	Signal to noise ratio
F1	Fundamental response
F2	First harmonic
F3	Second harmonic
ERG	Electroretinogram
BMI	Body mass index
OFC	Occipitofrontal circumference
DEPCAT	Carstairs deprivation index
LBW	Low birth weight
SMA	Small for gestational age
ISCEV	International Society for Clinical Electrophysiology of Vision
GQ	General Quotient
SD	Standard deviation
IQR	Inter-quartile range
NNU	Neonatal unit
VA	Visual acuity

Units

ms	milliseconds
µV	microvolts
cm	centimetres
gm	grams
Hz	Hertz
cds/m^2	candela seconds per metre squared
1’	1 minute of arc

Summary

Background / Aims:

Maternal drug misuse in pregnancy is a significant clinical and public health problem. Consequences for the newborn infant include prematurity, intrauterine growth restriction (IUGR) and neonatal abstinence syndrome (NAS). There is increasing evidence that maternal drug misuse in pregnancy may have longer term adverse effects on infant visual and neurodevelopmental outcome. Most of the evidence regarding visual outcomes in particular derives from small uncontrolled studies with a lack of adequately powered, controlled studies to date.

The visual evoked potential (VEP) can be used to assess the integrity and maturity of the infant visual pathway and both visual and neurodevelopmental abnormalities can be predicted by abnormal VEPs in infancy. Drug misuse is also associated with alteration of the VEP in adults and in animal models. Many drugs used in pregnancy can cross the placenta and enter the fetal circulation, including illicit drugs and prescribed methadone, which is the currently recommended treatment for pregnant opiate-dependent women. Hitherto few studies have investigated the effects of maternal drug misuse upon the newborn infant VEP.

This study investigates in detail the effects of prescribed methadone and additional illicit drug use in pregnancy upon the infant VEP recorded at birth and at six months of age, and explores any association with NAS. The range and incidence of visual and neurodevelopmental abnormalities at six months of age is described, and how these relate to a history of NAS and the pattern of *in utero* drug exposure is explored.

Pilot work:

Pilot work demonstrated the feasibility of recording neonatal flash VEPs in a small group of infants exposed to methadone *in utero,* and showed that drug exposed infants had abnormal VEPs compared to unmatched controls.

A further pilot study described longer term visual outcomes, which included nystagmus, reduced visual acuity and strabismus, in a selected group of infants and children exposed to methadone *in utero*, thus informing clinical and electrophysiological assessment at six months of age. The pilot studies were followed by a major prospective cohort study.

Prospective Study:

One hundred and two term infants of mothers prescribed substitute methadone during pregnancy and 50 comparison infants matched for birth weight, gestation and socio-economic group were recruited in the neonatal period. Flash and flicker VEPs were recorded from the occipital scalp of infants within three days of birth. Drug exposure was determined by maternal history, maternal and infant urine and meconium toxicology. Excess alcohol exposure *in utero* was determined by elevated fatty acid ethyl esters in meconium.

Neonatal flash VEPs were classified as *mature*, *typical*, or *immature* according to waveform morphology, and amplitude and latencies measured. Flicker VEPs were analysed using a fast-Fourier transformation and responses at each flicker frequency determined.

The same cohort of drug-exposed and comparison infants was invited for clinical visual evaluation at six months of age in conjunction with pattern-onset VEPs and Griffiths developmental assessment.

Results:

Neonatal testing:

Neonatal VEPs were successfully recorded from 100 drug-exposed infants and 50 matched comparison infants at a median age of 24 hours (IQR 13-44). Gestational age, birth weight and socio-economic group did not differ between groups. Flash VEPs from methadone-exposed infants had fewer P1 components ($p=0.001$), and were more likely to be of immature waveform ($p<0.001$) compared to comparisons. VEPs from methadone-exposed infants were also smaller in overall amplitude (median 27μV *vs* 39.5μV, $p<0.001$). The relative risk

of an abnormal VEP in the methadone-exposed cohort was 5.6 with an attributable risk percent of 82%. The majority of infants were exposed to illicit drugs in addition to prescribed methadone, most commonly opiates (74%) and benzodiazepines (66%). VEPs did not differ between infants exposed to opiates only, those additionally exposed to benzodiazepines and those exposed to stimulants. Regression analysis confirmed that the difference in VEP parameters between drug-exposed and comparison infants was associated with methadone exposure and not other drugs of misuse.

48% of the methadone-exposed cohort developed NAS requiring pharmacological treatment; there was no association between neonatal VEPs and subsequent onset or severity of NAS.

Flicker VEP analysis demonstrated an optimal flicker frequency of 4.6 Hz in both groups, but there were few differences in the proportion of responses between groups.

Six month follow-up:

Retention rate to six month follow-up was 79% for the methadone-exposed cohort and 52% for comparison infants. Age at assessment (median 27 weeks, range 26-30 wk), weight and OFC did not differ between groups. The demographic characteristics of comparison infants who were followed up were compared to those of comparison infants who were not followed up. There were no significant differences in birth weight (2 sample t-test p=0.445), OFC (2 sample t-test p=0.712), gestation (Mann-Whitney test p=0.984), 5-minute Apgar score (Mann-Whitney test p=0.263) or DEPCAT score (Mann-Whitney test p=0.258) between groups.

Methadone-exposed infants were more likely to have visual abnormalities than comparison infants, even after correcting for excess *in utero* alcohol exposure (40% *vs* 8%; adjusted p=0.007). Abnormalities in the methadone-exposed cohort included nystagmus (11%), strabismus (25%) and reduced visual acuity (22%). The relative risk of an abnormal visual outcome in the methadone-exposed cohort was 5.1 with an attributable risk percent of 80%.

Electrophysiological abnormalities persisted at six months of age: methadone-exposed infants had smaller amplitude pattern VEPs (25 μV *vs* 34 μV; p=0.005) with delayed peak latencies (115ms *vs* 99ms; p=0.019) and fewer responses at the small check size (p=0.003), compared to controls.

Methadone-exposed infants had significantly lower neurodevelopmental scores compared to comparison infants (GQ 97 for cases *vs* 105 for controls; p<0.001), even after correcting for maternal smoking, antidepressant treatment and excess alcohol consumption during pregnancy. Infants exposed to poly-drug misuse and treated for NAS in the newborn period performed particularly poorly on their neurodevelopmental scores. Visual impairment was an independent predictor of poor neurodevelopmental outcome and most infants scoring <85 on neurodevelopmental assessment had co-existing visual problems.

Conclusions:

In utero exposure to prescribed methadone and other substances of misuse is associated with an alteration in visual electrophysiology in the newborn period suggestive of immature visual maturation. These changes are independent of additional benzodiazepine or stimulant exposure, and appear to be associated with prescribed substitute methadone.

At six months of age, there is a high incidence of clinical visual abnormalities in infants exposed to methadone and other drugs of misuse *in utero*. Persistence of electrophysiological abnormalities beyond the neonatal period suggests that opiates may have a longer term effect on the developing visual system. Drug-exposed infants also have poorer neurodevelopmental scores than matched comparison infants after correcting for maternal smoking and excess alcohol intake. The bias of loss to follow-up was minimised by the high retention rate of drug-exposed infants. Although there was a higher loss of comparison infants, there were no differences in demographic characteristics between comparison infants followed up and those not followed up, suggesting the groups were similar. In addition, published data suggest the incidence of visual abnormalities described in the comparison population to be representative of the larger population. Infants born to drug-misusing mothers are highly vulnerable and warrant early comprehensive visual assessment.

1 Chapter 1 Introduction

1.1 Drug misuse in pregnancy

1.1.1 Incidence

The use of illicit non-prescribed drugs by pregnant women is unfortunately a common problem in some parts of the United Kingdom (UK) (1-5). There are thought to be between 250,000 and 350,000 children of known problem drug users in the UK - about one child for every problem drug user (6). In Scotland it is estimated that 4-6% of all children under the age of 16 years have a drug-using parent, representing between 41,000 and 59,000 children. The incidence of maternal drug misuse is increasing with anonymous screening suggesting that 11-16% of expectant women use at least one illicit substance during pregnancy (7). It is also well recognised that a high percentage of women misuse more than one drug in pregnancy - studies have shown that the majority of mothers prescribed methadone in pregnancy misuse other substances, with up to 66% taking additional benzodiazepines and heroin (8,9).

1.1.2 Pregnancy outcomes

Among the drug misusing population the incidence of unplanned pregnancy is high and antenatal care is often erratic. Substance misuse in pregnancy is associated with increased risk of pregnancy complications including premature rupture of the membranes, placental abruption, antepartum haemorrhage, stillbirth and neonatal death (1,2). Infants born to drug misusing mothers represent a very high risk group, with increased rates of preterm birth and intra-uterine growth restriction (IUGR) (1,5,10-12). Substance misusing mothers as a group tend to suffer from the consequences of poverty including physical and mental ill health and poor nutritional status. They are likely to smoke cigarettes, and may experience domestic violence and drink excessive amounts of alcohol (1,13). Such unfavourable circumstances pose a threat to the health of the newborn, not least to neurological and visual development.

1.1.3 Management in pregnancy

Management of maternal opiate misuse during pregnancy includes substitute prescribing of methadone, a synthetic opioid which stabilises lifestyle, reduces the incidence of preterm birth and IUGR and reduces risk-taking behaviour (2,5,10-12). Several studies have reported an increase in birth weight and reduction in neonatal mortality rate associated with prescribed methadone use compared to illicit opiates. Meta-analysis found the mean reduction in birth weight below normal with maternal methadone use to be 279 grams, compared to a reduction of 489 grams with illicit heroin use (14). The use of methadone in pregnancy is associated with better compliance with antenatal obstetric care and better preparation for parenting responsibilities (11). Regular prescription of methadone engages patients and facilitates attendance for both antenatal care and social service support. Methadone is also more pharmacologically stable than heroin: it is more slowly absorbed and longer acting and leads to stable blood concentrations when taken daily. Maintenance methadone abolishes most of the symptoms of heroin intoxication and withdrawal, which are harmful to the fetus. Attempted withdrawal from illicit opiates and methadone treatment during pregnancy has poor outcomes and guidelines recommend that opiates should not be withdrawn after 32 weeks' gestation (13).

Despite the potential benefits, substitute prescribing of methadone in pregnancy remains an emotive topic, which has attracted political and media debate and led to some health professionals' refusal to prescribe (15). One significant disadvantage of methadone is the high incidence of neonatal abstinence syndrome (NAS) which is seen in infants who have been exposed to methadone *in utero*.

1.1.4 Identification of drug exposure

1.1.4.1 Techniques

Maternal reporting of drug misuse is often inaccurate due to guilt, embarrassment and/or fear of legal or custodial repercussions. Maternal interview has been found to have a low sensitivity for detecting opiate and cocaine exposure (67% and 65% sensitivity respectively) (16). As a result of this,

various techniques to determine more accurately *in utero* drug exposure have been established. In clinical practice drug screening is performed as infrequently as possible to foster a sense of trust and responsibility and to keep women engaged with health services (8). In research practice, however, additional toxicology analysis is often employed to determine a more comprehensive pattern of drug exposure. Techniques for detecting gestational exposure to drugs of misuse include maternal and infant urine or blood analysis, meconium and neonatal hair analysis.

1.1.4.2 Blood/urine

Blood samples for methadone levels can be taken from the umbilical cord after delivery or by either venesection or capillary blood sampling of the infant. Urine toxicology can detect the vast majority of drugs of misuse and is easily performed on both mother and infant postnatally. The main limitation of both blood and urine analyses is that due to the short half-life of many drugs of misuse, these methods can only be used to detect drug exposure late in pregnancy. Cocaine in particular has a very short half-life and can only be detected in blood or urine for up to one week following exposure. Cannabis and opiates have longer half-lives but will still only be detected for a maximum of three to four weeks following exposure. These limitations have led to the development of other biological markers, which reflect longer term exposure to illicit drugs and alcohol.

1.1.4.3 Meconium/hair

Meconium and neonatal hair analysis have become established methods for defining *in utero* exposure to drugs of misuse (17,18). Deposition of drugs into meconium begins at approximately 12 to 16 weeks of gestation when fetal swallowing commences, and therefore meconium analysis examines drug exposure in the second two trimesters of pregnancy. Neonatal hair grows during the third trimester and will reflect exposure to illicit drugs during this time. Bar-Oz *et al* (2003) investigated the sensitivity of meconium analysis and neonatal hair analysis in 185 paired samples collected from infants with a history of *in utero* drug exposure (17). They found that meconium analysis was more sensitive at detecting cocaine and cannabis compared to neonatal hair analysis and, since

meconium is a discarded material, had the added advantage of being more acceptable to parents. All drugs of misuse commonly screened for in infant urine can be detected by meconium analysis including opiates, methadone, benzodiazepines, cocaine, cannabinoid and amphetamine.

Meconium analysis can also be used to detect prenatal ethanol exposure (19,20). As prenatal ethanol exposure may be a confounding factor for abnormal visual and neurological development, it is important to measure co-exposure in studies of drug-exposed infants. In a similar manner to under-reporting of illicit drug use, pregnant women will often deny excessive alcohol use during pregnancy and a biological marker of exposure would therefore be useful.

Ethanol conjugates to a number of fatty acid species collectively named fatty acid ethyl esters (FAEEs). Ethanol can cross the placenta in pregnancy due to its small molecular size and high water solubility and enter the fetal circulation. Fetal ethanol metabolism leads to the formation of FAEEs, which are deposited in fetal meconium. Studies have shown that meconium FAEE analysis is fivefold more sensitive than self-reported screening for detection of ethanol-exposed pregnancies (19).

1.2 Effects of maternal drug misuse on the infant

1.2.1 Neonatal abstinence syndrome

Illicit substances used by mothers in pregnancy can cross from the maternal circulation, via the placenta to enter the fetal circulation, resulting in a physical drug dependency in the newborn. At birth division of the umbilical cord leads to abrupt discontinuation of the supply of illicit drug to the infant, which can result in neonatal drug withdrawal commonly referred to as neonatal abstinence syndrome (NAS). Signs of NAS include irritability, jitteriness, increased muscle tone, poor feeding, tachypnoea, diarrhoea, sweating, sneezing, yawning, skin excoriation and, in extreme cases, convulsions.

Methadone, morphine and heroin activate opiate receptors in the brain, which decrease the activity of adenylate cyclase, resulting in reduced cAMP production and release of noradrenaline. During chronic exposure, noradrenaline release gradually increases towards its normal level as tolerance to the drug develops. If opiates are withdrawn, their inhibitory effect is lost, resulting in symptoms of withdrawal.

Signs of NAS may develop within 12 hours of birth or they may not be apparent until the second week of life or even later. This is due to differences in the pharmacological properties of addictive substances and/or differences in metabolism. Approximately 40-60% of infants who have been exposed to methadone *in utero* will develop symptoms of NAS (8,12,21,22).

Factors that may influence the development of NAS include maternal methadone dose, the use of other illicit drugs, maternal cigarette smoking, rate of placental transfer of methadone, inter-individual variation in metabolism of methadone between mother and baby, and breast-feeding (8,9,23-25).

Prescribed maternal methadone dose has not consistently been found to correlate with the development of NAS. Some of the studies investigating this did not, however, correct for confounding factors such as additional illicit drug use (12,22). A large recent retrospective audit of 450 singleton infants born to mothers prescribed methadone in pregnancy showed a strong correlation

between prescribed maternal methadone dose and the risk of the infant developing NAS, even when corrected for additional illicit drug use (8). Heavy cigarette smoking concurrent with methadone use is associated with higher NAS scores (23), and use of illicit drugs in addition to methadone may increase the requirement for treatment (9). Breast-feeding appears to reduce the requirement for pharmacological treatment of NAS (8,26): Dryden *et al* found that breast-feeding for greater than 72 hours was independently associated with a halving of the odds of the infant requiring pharmacological treatment for NAS. The mechanism of this is unclear, as studies have demonstrated that transfer of methadone into maternal milk is minimal (25). It may partly be due to the soothing effect that breast-feeding has on newborn infants (27).

Several objective scoring systems exist to guide initiation and intensification of treatment; the most commonly utilised of these are the Finnegan and Lipsitz scores (28,29). Treatment options for infants developing NAS secondary to maternal opiate use in pregnancy include opiates, sedatives (phenobarbital or diazepam) and supportive treatments (swaddling, pacifiers, massage, relaxation baths, and waterbeds) (30-33). Cochrane meta-analysis of available studies found a reduction in treatment failure with opiate use compared to the other interventions (30). A combination of opiate and phenobarbital may reduce the severity of withdrawal and duration of hospitalisation as well as improving neurobehavioural scores, and is often required to treat NAS following polydrug misuse (32,33).

Due to the many different factors involved in the development of NAS, it is impossible to predict the likelihood of onset in individual cases and so the management of infants born to drug-misusing mothers is expectant, usually involving prolonged postnatal hospital stay to observe for signs of NAS. One study reported that the median postnatal stay for healthy maternal drug-exposed infants who did not require pharmacological treatment was seven days, more than three times longer than the median stay for healthy non-maternal drug-exposed babies (8). This extended postnatal stay does however have some advantage in allowing for social work assessment prior to discharge and organisation of a comprehensive post-discharge care plan.

Infants born to drug misusing mothers constitute a significant workload to health and social services and are a resource burden: Dryden *et al* found that infants born to mothers prescribed methadone in pregnancy accounted for 2.9% of all hospital births but 18.2% of neonatal cot days. Better understanding of the patho-physiology of NAS would be helpful in managing drug misuse in pregnancy and guiding treatment of infants.

1.2.2 Neurodevelopmental outcomes

There are numerous data linking maternal opiate misuse with developmental delay (34-50). Fifteen studies have followed up a total of 770 drug-exposed infants and reported on their neurodevelopmental outcome at ages ranging from two months to 12 years. Various different scales of infant and child development were used and, due to heterogeneity of the studies, meta-analysis is not possible. Furthermore, retention to follow-up was often low (a mean of 66% in infants followed up after one year of age). Motor developmental delay was demonstrated in several of the studies (34,38,49) and a low mental development index or low intelligence quotient (IQ) was found by many authors (34,35,38,43,47). Several papers report behavioural problems in children who had been exposed to drug-misuse *in utero,* including aggression, poor concentration, social inhibition and, in particular, attention deficit hyperactivity disorder (44-46,48,50). Small head circumference is commonly reported at birth, usually associated with low birth weight, although catch-up growth does occur. Other reported neurological anomalies include cerebral palsy and abnormalities of muscle tone and posture (34,45,47).

Environmental factors and poor parenting skills may in part account for some of these findings. Attempts have been made to correct for confounding factors by examining the differences in outcome in children raised by their parents compared to those who had been adopted (45). One study found that children in adoptive homes had better psychomotor scores than those living with their parents, suggesting that the environment plays a significant role in outcome. The children in adoptive homes, however, still had significantly lower scores on one of their performance scales compared to non-drug exposed controls (45). Bunikowski *et al* (1998) found no difference in developmental outcome between

infants in foster care versus those living with their biological parents, and described significantly lower development quotients in drug-exposed infants compared to controls (34). These studies are not necessarily contradictory as children in foster care are commonly moved between accommodations including those of their natural parents. Topley *et al* (2008) described an optimistic outcome in children of drug-misusing parents who were in full-time schooling, with no child having a statement of special educational need and a similar number (17%) requiring a low level of additional support in school compared to the corresponding local population (48). None of the children in this latter study had a formal developmental assessment performed however, and a high proportion had behavioural or concentration difficulties.

The high loss of study subjects to follow-up is a criticism of many of these studies and is a reflection of the social disruption that is associated with the drug culture. It is unlikely, however, that infants whose family life is so chaotic that they are untraceable at follow-up will perform better than those infants for whom data are obtainable (38). The retention of infants in longitudinal or follow-up studies from socially deprived backgrounds is recognised to be challenging. However various techniques can be employed to maximise study retention rates including reimbursement of transport costs and diligent tracking of participants (51).

1.2.3 Visual outcomes

Prenatal exposure to various harmful substances can have adverse effects on infant visual development (52). Strabismus, nystagmus, hypoplastic optic discs, delayed visual maturation and prolonged eyelid oedema have been reported following *in utero* cocaine exposure, as well as both superficial and deep retinal haemorrhages (53-55). It is postulated that these findings may be due in part to retinal vascular changes caused by the vasoconstrictive effects of cocaine on placental blood vessels.

Ocular abnormalities are also seen in infants with fetal alcohol syndrome, and include short horizontal palpebral fissures, epicanthus, telecanthus, microphthalmia, refractive errors, strabismus and retinal vessel tortuosity (56-58). Up to half of infants born to alcoholic mothers demonstrate optic nerve

hypoplasia. This is consistent with animal studies: *in utero* alcohol exposure in rats is associated with ultra structural damage to the macroglial cells and myelin sheaths and hypoplasia of the optic nerve (59,60). Animal models demonstrate that ethanol exposure during a critical period of early development has an adverse effect on neurotransmitter systems, resulting in apoptosis of developing neurons. A similar pattern of apoptotic neurodegeneration may lead to many of the ophthalmic manifestations and other central nervous system features of fetal alcohol syndrome.

There are fewer data regarding visual outcome in infants exposed to opiates and/or benzodiazepines *in utero*. Gill *et al* (2003) undertook ophthalmology assessment in 49 infants born to opiate-dependent mothers, the majority of whom (77%) were exposed to opiates alone (61). Twenty-nine infants had a full examination and 20 completed a telephone questionnaire only. Seven of the infants examined had confirmed strabismus and a further six of the telephone-surveyed children had a history of intermittent strabismus. This equates to an incidence of strabismus of over ten times that of the general population. The authors found no differences in the incidence of strabismus between those infants treated for NAS and those not (61). Similarly, Nelson *et al* (1987) found an incidence of strabismus of 24% in their cohort of 29 infants assessed up to two years of age (62). The majority of infants in this study were however exposed to polydrug misuse including cocaine and amphetamines. A recent Scottish study found that 26% of infants born to opiate-using mothers failed health visitor vision screening tests in the community on at least one occasion within the first six months of life: 42% of the referred group had abnormalities confirmed by formal ophthalmology assessment including nystagmus and squint (4).

The presence of nystagmus in five children born to drug-addicted mothers was reported first in 2002 (63). Three children presented with congenital horizontal pendular nystagmus and two children with a transient horizontal nystagmus in association with NAS. More recently nystagmus, strabismus and delayed visual development were described in 14 infants exposed to methadone and/or benzodiazepine *in utero* (64). The nystagmus was horizontal in all cases and in most cases was noted in the first six months of life. Recent follow-up of this cohort has suggested the nystagmus and reduced visual acuity may be permanent in children exposed to opiates and benzodiazepines *in utero* (65).

1.3 Assessing the infant visual system: clinical assessment

The most common visual abnormalities reported in infants who have been exposed to opiate misuse *in utero* are reduced visual acuity, nystagmus, delayed visual maturation, strabismus and refractive errors (61,62,64,65).

Visual acuity can be assessed in infancy using Cardiff or Teller acuity cards (66). Cardiff cards contain simple picture images and Teller cards contain black and white grating patterns. Cardiff picture images are drawn with a white band bordered by two narrow black bands, all on a neutral grey background. If the visual target lies beyond the subject's acuity limit it merges with the background and becomes invisible. The picture targets are of the same overall size but with decreasing width of black and white bands: acuity is defined by the narrowest white band for which the target is visible. The principle of the test is preferential looking – an infant will choose to look towards a visible target rather than a plain stimulus.

Nystagmus described in association with maternal drug misuse is usually horizontal in nature and will be detected by observation (64,65). Manifest nystagmus is apparent on observation of the child with both eyes open, and is usually best observed with the child fixating on a small toy. Latent nystagmus is a type of congenital nystagmus that is only present with monocular viewing, and manifest latent nystagmus is that which is present with both eyes open but beats in a different direction depending on which eye is viewing (i.e. always towards the viewing eye).

Delayed visual maturation (DVM) is the condition whereby infants appear behaviourally visually delayed at a young age with no corresponding ocular or central nervous system abnormalities, but then recover vision over a period of time (usually by six months of age) (67,68). Such infants usually present in the first few months of life with a failure to fix and follow and will demonstrate reduced visual acuity on testing. The diagnosis is made retrospectively when the infant recovers normal vision.

Strabismus (squint) can be detected using the Hirschberg test in infancy (equal pupillary light reaction) (69). The presence of strabismus in an infant over six months of age warrants ophthalmology assessment. Timely detection can lead to early intervention with correction of refractive errors, selective patching and occasionally surgery (69). Unattended strabismus can lead to amblyopia, susceptibility to which is greatest in the first three years of life. In addition, the cosmetic disorder associated with strabismus can interfere with social and psychological development: children with uncorrected strabismus are at significantly greater risk of displaying conduct and externalising problems (70).

Refractive errors require retinoscopy for diagnosis and include hypermetropia (long sightedness), myopia (short sightedness) and astigmatism. Accurate assessment of refractive errors in infancy usually requires pupil dilatation as young infants will not fixate on a static object for assessment. The technique of rapid retinoscopy through undilated pupils has been described as an efficient method of detecting high refractive errors and candidates for nonstrabismic amblyopia in childhood (71).

Cerebral visual impairment (CVI) involves disordered higher visual processing and may also involve reduced visual acuity and visual fields (72-74). CVI can be diagnosed by structured history taking and validated by observation of behaviour and testing. Two higher visual processing pathways have been described: the dorsal stream and the ventral stream. The dorsal stream passes between the occipital lobes and the posterior parietal lobes and allows appraisal of the whole visual scene and visual guidance of movement through the scene. Dorsal stream dysfunction causes problems with the processing of complex visual scenes such as finding a toy in a toy-box or on a patterned background, finding clothes in a drawer and seeing objects at a distance (as there is more to see). It also causes problems with visual guidance of movement such as inaccurate reach and grasp, difficulty with curbs and steps, and difficulty crossing floor boundaries. The ventral stream passes between the occipital lobes and the temporal lobes and is responsible for visual recognition, orientation and visual memory. Ventral stream dysfunction causes problems with recognising faces, understanding facial expression, navigation and copying. Children with CVI will often develop coping strategies to help them adapt to everyday life, such as coding and recognising by colour. Problems interpreting the visual world and the compensatory strategies

required can affect behaviour; some children demonstrate hyperactivity, withdrawal and aggression. The implementation of a developmental programme and strategies to modify behaviour can result in significant improvement in visual function (72-74).

1.4 Assessing the infant visual system: visual evoked potentials

The visual evoked potential (VEP) is an electrical signal generated in the visual cortex of the brain in response to a visual stimulus (75,76). A normal VEP depends upon an intact visual pathway from the retina via the optic nerves and optic chiasm to the lateral geniculate nuclei and visual cortex (Figure 1-1).

VEPs reflect visual and cortical integrity and are a useful measure of visual development. They can be used to detect, quantify and monitor abnormalities of the visual system (76). In clinical paediatric practice, VEPs are used in the detection and management of optic nerve hypoplasia or atrophy, amblyopia, congenital cataract, delayed visual maturation and cortical visual impairment (76). An abnormal VEP is strongly predictive of visual abnormalities and adverse neurological outcome in selected preterm and term infants (77-81).

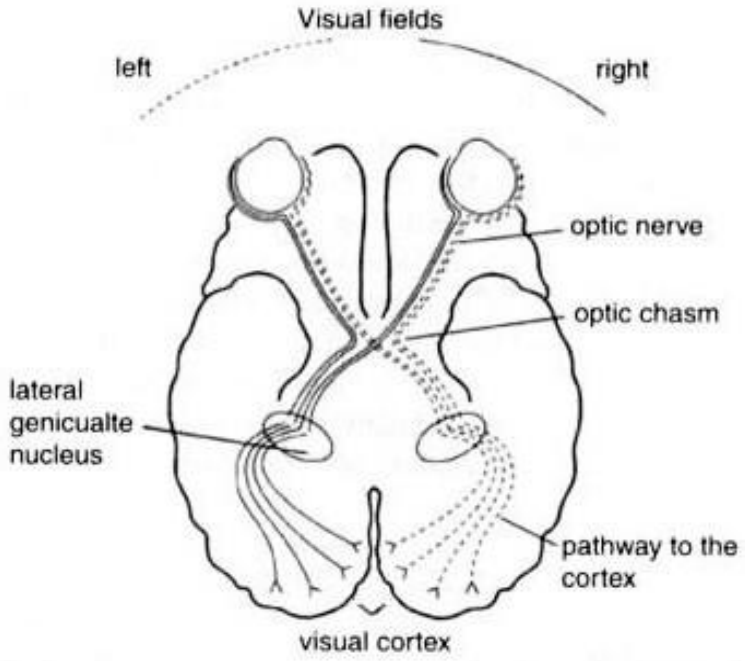


Figure 1-1 Visual pathways in the brain

1.4.1 Types of VEPs

The visual stimuli used to elicit VEPs can be luminance or pattern (75). Luminance stimulation involves either a flashing or flickering light source. The flash VEP is elicited by a light source with a relatively long inter-stimulus interval and is classified as a transient VEP. The flicker VEP is elicited by rapid repeated visual stimulations that overlap in time producing a continuous oscillating waveform and these are classified as steady-state VEPs. The pattern stimulus is commonly a black and white checkerboard or black and white grating, usually presented on a high resolution computer monitor.

1.4.2 The infant VEP

1.4.2.1 Flash VEP

The flash VEP is most commonly used in the newborn period, as it does not require visual fixation. The flash VEP waveform is characterised by a series of deflections designated as negative and positive in a numerical sequence (N1, P1, N2, P2, N3, P3) (Figure 1-2). Each negative and positive deflection can be described in terms of its latency (time from the stimulus onset to the corresponding deflection in milliseconds (ms)) and its amplitude. The amplitude of each deflection is measured in microvolts (μV) and may be described either from baseline or between peaks and troughs.

All healthy term newborns should demonstrate a flash visual evoked response. A positive waveform at approximately 200ms (P2) has been found to be the most consistently present component, found in 100% of newborns by Benavente *et al* (2005) and 94% by Shepherd *et al* (1999) (78,82). A negative component at approximately 300ms (N3) was present in 82% of term infants tested by Shepherd and in 42% of infants tested by Benavente. Both these studies measured flash VEPs in healthy term newborn infants within the first five days of life.

Over the first few months of life the VEP waveform becomes more complex and a positivity emerges at approximately 100ms (P1) (Figure 1-3). The latencies of P1 and P2 components decrease with increasing postnatal age so that by six months of age the mean latency of P1 has reduced from 102ms to 81ms and the

mean latency of P2 has reduced from 200ms to 147ms. The amplitude of both P1 and P2 increases significantly with increasing postnatal age and by six months of age the flash VEP has matured significantly and starts to resemble that of an adult (82,83).

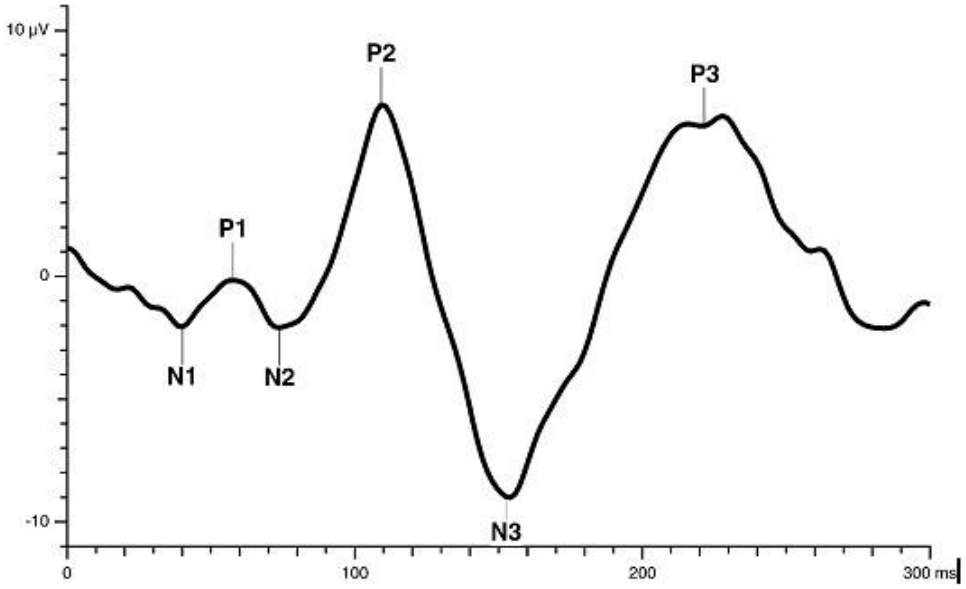


Figure 1-2 Adult flash VEP
Six components are present (N1, P1, N2, P2, N3, P3). The major component, P2 has a latency of approximately 120ms in healthy adults.

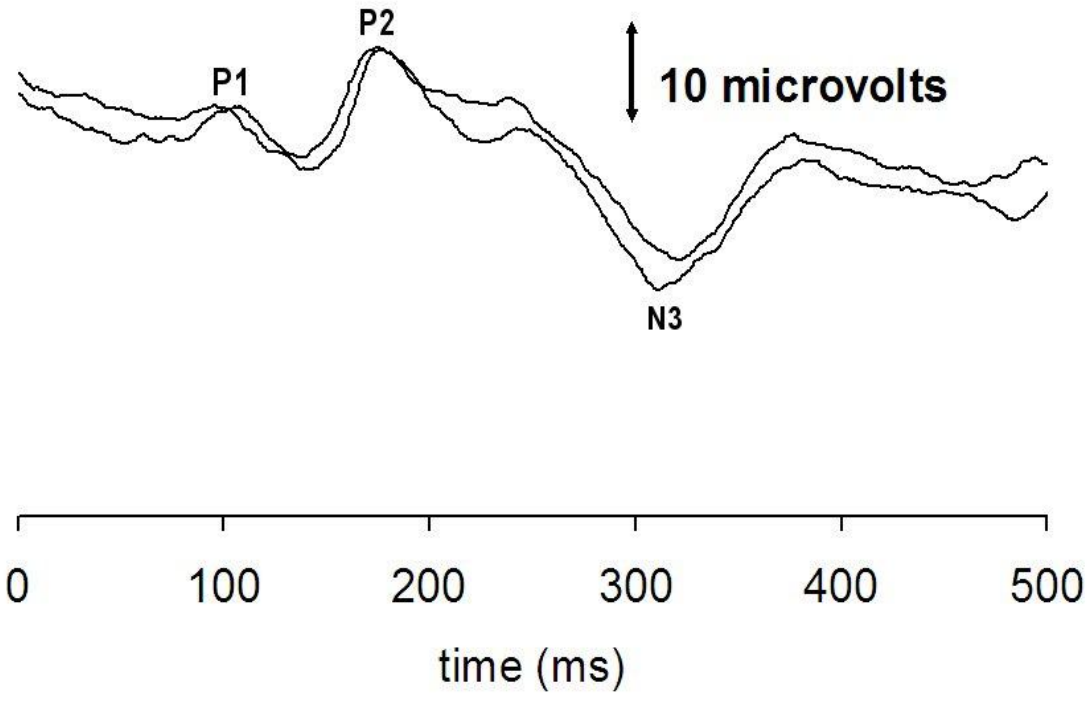


Figure 1-3 Infant flash VEP
Components present include positivity at 100ms (P1), positivity at 200ms (P2) and negativity at 300ms (N3).

1.4.2.2 Flicker VEP

An alternative and novel luminance electrophysiology technique is the flicker VEP. A flickering light stimulus will elicit VEPs that overlap in time to produce a continuous oscillating waveform. A mathematical technique, the Fourier analysis, is used to interpret the data (84,85).

As with the flash VEP, the flicker response does not require infant visual fixation and can therefore be recorded in the newborn period. Early data suggest that the flicker response may provide an alternative practical and objective indicator of visual pathway integrity (86-88). The flicker VEP involves presenting a flickering light stimulus at various frequencies usually ranging from 4 to 40 Hz. This flickering light stimulus has the potential to evoke cortical activity at the stimulation frequency. Mathematical analysis of the VEP by Fourier transformation will determine whether a response is present at the frequency under investigation (Figure 1-4). A cortical response detected at the exact stimulus frequency being applied is designated the fundamental response (F1). A cortical response present at an exact multiple of the stimulus frequency is designated the harmonic response (F2 at double the stimulus frequency, F3 at triple the stimulus frequency). The optimal stimulus frequency is the frequency which generates the largest amplitude response. Limited data available in the literature suggest the optimal flicker frequency is approximately 4Hz at one week of age, increasing to 7Hz at six months of age, still considerably below the optimal flicker frequency of approximately 18Hz in adult subjects (87,88).

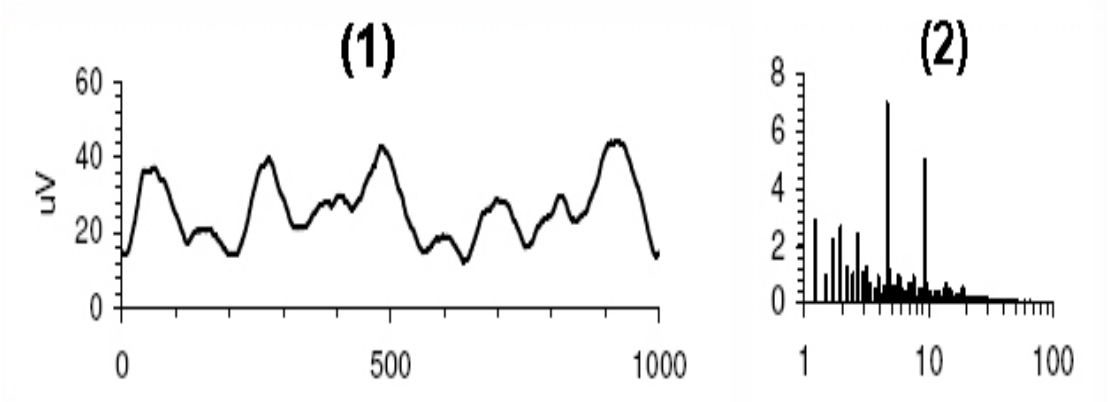


Figure 1-4 Infant flicker VEP
A 4.7 Hz flickering light stimulus produces a continuous oscillating waveform, which is shown in (1). Fourier analysis is applied to the data to convert it from the time domain (1: time on x axis in milliseconds) into the frequency domain (2: frequency on x axis in Hz). This demonstrates a response at 4.7 Hz (large amplitude spike at a frequency of 4.7Hz on graph (2)). The second large spike at approx 10 Hz is the harmonic response (2). A harmonic response is commonly seen in analysis of the flicker VEP although its aetiology is unclear. It is signified by a response at an exact multiple of the primary response – in this case the harmonic response in graph (2) is shown by the large spike at 9.4Hz.

1.4.2.3 Pattern VEP

The recommended pattern stimulus is a black and white checkerboard (75). The stimulus is described by the visual angle subtended by the side of a single check and measured in degrees and minutes of arc subtended at the eye. One minute of arc (1') is a unit of angular measurement equal to one sixtieth (1/60) of a degree.

Table 1-1 Pattern VEP check sizes

Minutes of arc	Degree	Size of check
15'	0.25	Small
60'	1	Medium
120'	2	Large

All checks should be square with an equal number of light and dark checks. Two forms of pattern VEP testing have been established – pattern reversal and pattern onset. Pattern reversal involves a pattern that abruptly reverses (i.e. black to white and white to black) at a specified number of reversals per second. In the pattern onset VEP, the pattern is abruptly exchanged with a diffuse grey background. Overall screen luminance must remain equal during pattern reversal or pattern onset/offset.

Visual stimulation with an alternating pattern produces a VEP waveform that in healthy adult subjects consists of a negative deflection at 75ms (N75), a positive deflection at 100ms (P100) and a negative deflection at 135ms (N135) (Figure 1-5). The pattern reversal VEP has relatively low intrasubject and intersubject variability and it is therefore the preferred method of testing in most circumstances. A pattern onset stimulus produces a similar negative-positive deflection designated C1 and C2 (Figure 1-6).

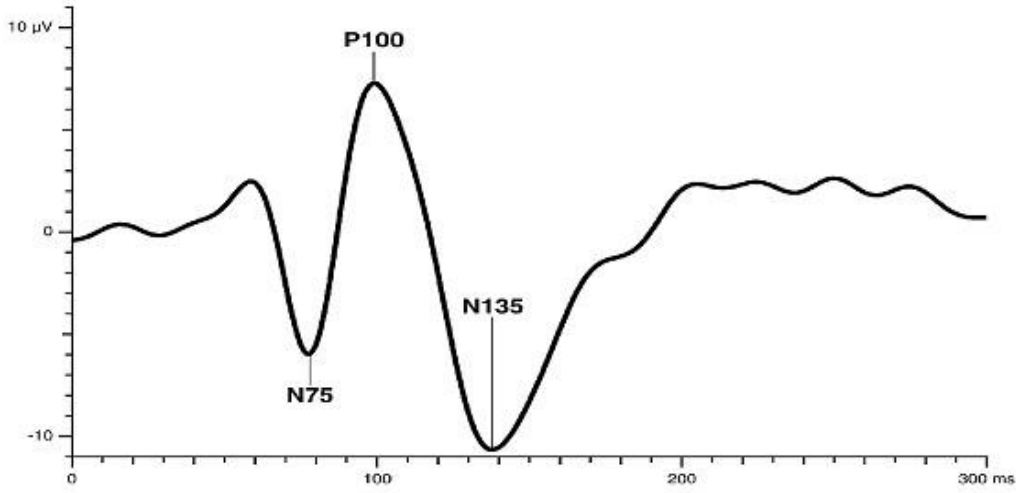


Figure 1-5 Adult pattern reversal VEP
The pattern reversal VEP typically consists of three components: a negativity at 75ms, a positivity at 100ms and a negativity at 135ms.

Figure 1-6 Adult pattern onset VEP
The pattern onset VEP is more variable in appearance than pattern reversal. The response consists of two major components in adults: a C1 component at approximately 75ms and a C2 component at approximately 125ms.

An assessment of cortical acuity can be made by reducing the spatial frequency of the pattern stimuli (i.e. the checks on the checkerboard get smaller and smaller) until no reproducible VEP can be recorded.

The pattern VEP is generally thought to have limited applicability in the newborn period, as it requires an awake and attentive baby. Harding *et al* (1989), however, have documented a positive component at 280 ms in response to a pattern reversal stimulus in infants between 33 and 37 weeks gestational age (89).

The pattern reversal VEP in healthy infants in the first year of life is well described; the main component being a simple positivity at approximately 200 ms, commonly referred to as P100 (Figure 1-7) (90-92). The VEP response to small checks is absent at birth but develops between two to four months of age. The latency of the P100 component reduces with increasing postnatal age, from approximately 200 ms at six weeks of age, to 150 ms at six months of age and 100ms at 12 months (92). Pattern VEP latencies are longer for smaller check sizes and therefore normative data specific for the check size under investigation must always be used. A study of pattern VEPs in 161 infants between three weeks and two years of age demonstrated rapid visual development in the first six months of life as shown by the development of reproducible VEPs to smaller check sizes and a rapid decrease in the latency of the first reproducible positive peak (92).

Infant fixation on the visual display should be monitored during pattern VEP testing and the recording interrupted during periods of loss of fixation. Various strategies may be used to direct the infant’s visual attention to the pattern screen and include dangling small objects in front of the screen and superimposing interesting pictures upon the pattern.

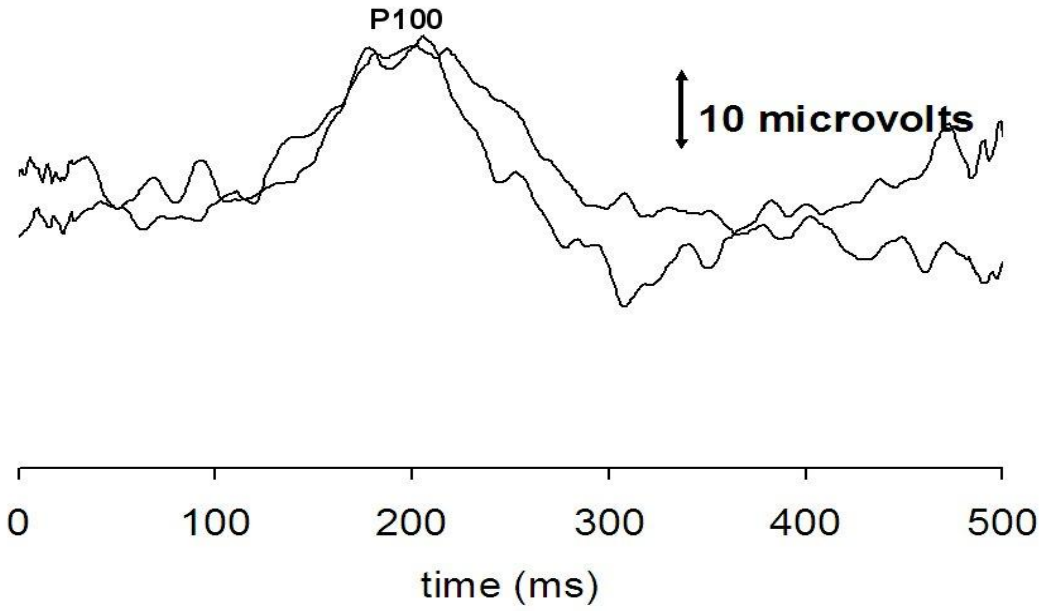


Figure 1-7 Infant pattern reversal VEP
Black and white reversing checkerboards produce a negative-positive-negative waveform, conventionally labelled N75-P100-N145: however, in infants, it is normal for the P100 peak to be as late as 200ms, depending on their age and the size of the checkerboard.

Summary: Characteristic features of a maturing VEP in infancy

Flash VEP:

Emergence of P2 component

Emergence of P1 component

Increased amplitude of components

Reduced latency of components

Flicker VEP:

Increase in optimal flicker frequency

Pattern VEP:

Reduced latency of components

Response detected at smaller check sizes

1.4.3 VEPs in preterm and sick infants

The flash VEP can be recorded from the occipital scalp as early as 24 weeks gestation. The dominant feature of the preterm flash VEP is a broad negativity at about 300 ms, commonly referred to as N3 (78,93). By around 35 weeks gestation the P2 component emerges and the VEP starts to resemble that of term infants (Figure 1-8).

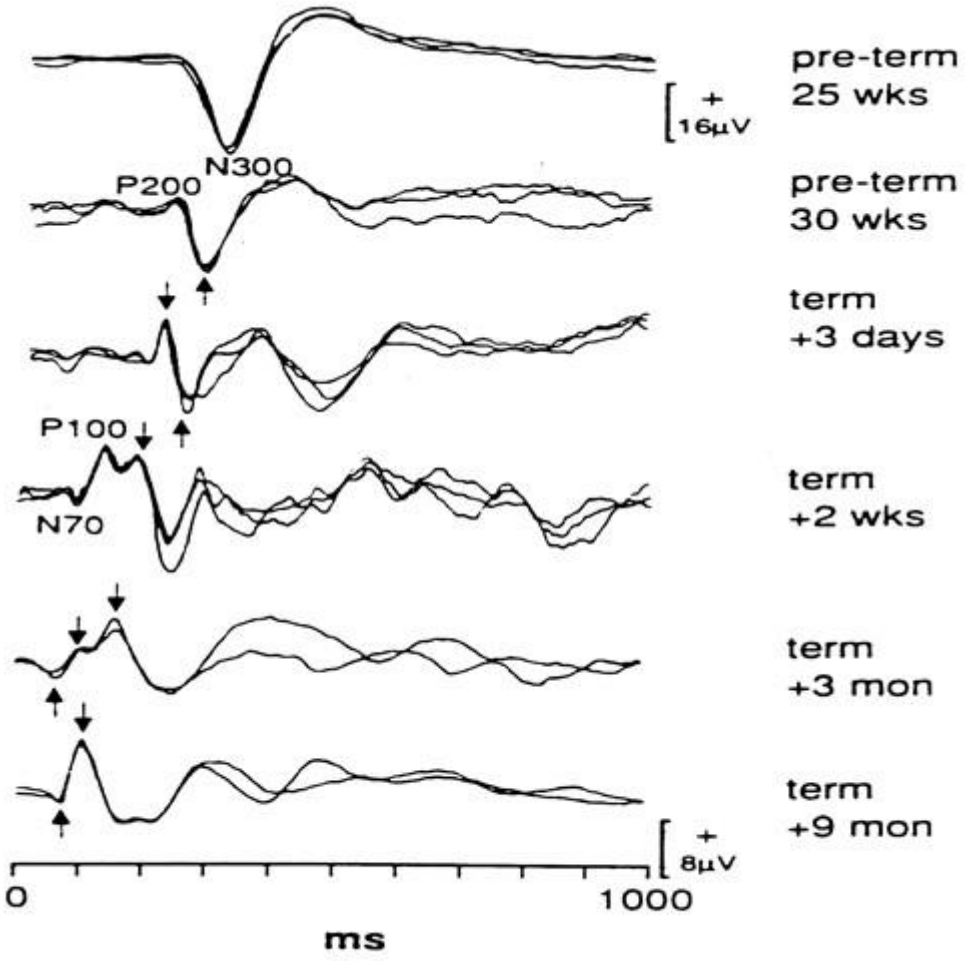


Figure 1-8 Maturation of the infant flash VEP
The predominant characteristic of the preterm infant VEP is a broad negativity at 300ms (N3). Taylor JM, McCulloch DL. Journal of Clinical Neurophysiology 1992; 9(3): 357-72.

Several studies have investigated the predictive value of the flash VEP in determining clinical outcome for preterm infants (78,94-97,77). Shepherd *et al* (1999) recorded the flash VEP in 81 preterm infants and found the sensitivity and specificity with regards to survival to be 86% and 89% respectively, and with respect to development of cerebral palsy to be 60% and 92%. Pike *et al* (2000) found the flash VEP could predict cerebral palsy with a sensitivity of 71% and specificity of 90% and Kato *et al* (2000) found corresponding values of 78% and 94% in their study of 60 preterm infants. Poor prognostic factors for outcome include an absent VEP at any age, a delayed N3 latency before term age and an absent P2 component at term. In their study of 62 preterm infants, Beverley *et al* (1990) found that an abnormal VEP correlated with intraventricular haemorrhage but was not predictive of neurodevelopmental outcome. Similarly, Ekert *et al* (1997) found that the flash VEP correlated with the development of periventricular leukomalacia but not with neurodevelopmental outcome.

The flash VEP has also been investigated as a tool to predict prognosis in term infants with perinatal asphyxia (79,98-100). The largest study by Taylor *et al* (1992) examined serial flash VEPs in 92 term infants with birth asphyxia. They found that death or severe neurological impairment could be predicted if an absent VEP was documented at any time or if abnormalities of the waveform persisted beyond day four of life. These findings were similar to other papers which have found VEPs to have a high predictive value for death or neurodevelopmental impairment (79,98,99).

Pattern orientation reversal VEPs have been described in term and preterm infants using rapid orientation reversal of sine wave gratings at 4 Hz and 8 Hz (101). The authors found that very low birth weight infants with normal neonatal ultrasound scans and neurological outcome had similar VEPs to term infants. Four infants in this study with abnormal cranial ultrasounds had absent VEPs and a subsequent abnormal neurological outcome (101). A further study by the same authors found the orientation reversal VEP to be predictive of a low neurodevelopmental score in preterm infants with a sensitivity of 86% and specificity of 65% (102). Mercuri *et al* (1999) found that the orientation reversal VEP also correlated with cerebral damage as assessed by MRI in term infants with perinatal brain insults (103). The use of VEPs in predicting outcome in preterm and term infants is summarised in Table 1-2.

Table 1-2 Predictive value of the VEP in infancy

Reference	VEP type	Subjects	Outcome	Sensitivity/specificity (where given)
Taylor, McCulloch (81)	Flash / pattern	32 children 4mth-5yr, cortical blindness	Predictive of long-term visual outcome	100% / 94 %
McCulloch et al (80)	Flash / pattern	25 term infants, birth asphyxia	Predictive of long-term visual outcome	-
Shepherd et al (78)	Flash	81 preterm infants	Predictive of death and CP	Death 86% / 89%, CP 60% / 92%
Pike, Marlow (97)	Flash	92 preterm infants	Predictive of CP and transient dystonia	71% / 90%
Kato et al (77)	Flash	60 preterm infants	Predictive of CP	78% / 94%
Taylor et al (100)	Flash	92 term infants, birth asphyxia	Predictive of death or severe neurological impairment	78% / 100%
Whyte et al (79)	Flash	25 term infants, birth asphyxia	Predictive of neurodevelopmental outcome	-
Muttitt et al (99)	Flash	36 term infants, birth asphyxia	Predictive of neurodevelopmental outcome	91% / 100%
Whyte (98)	Flash	93 term infants, birth asphyxia	Predictive of neurodevelopmental outcome	89% / 100%
Atkinson et al (102)	OR-VEP	26 preterm infants	Predictive of Griffiths development quotient < 80	86% / 65%
Mercuri et al (103)	OR-VEP	29 term infants, HIE or brain lesions on MRI	Predictive of neuromotor outcome	90% / 87%

CP: cerebral palsy, HIE: hypoxic ischaemic encephalopathy, MRI: magnetic resonance imaging

1.4.4 Clinical applications of the VEP

Visual acuity estimation:

VEP estimates of visual acuity are useful in infants who cannot co-operate with standard visual acuity tests (76,104). Visual acuity can be estimated by determining the smallest pattern size that elicits an identifiable VEP. With adequate co-operation reproducible VEPs should be obtained to check sizes down to 15 minutes of arc or smaller in children over three months of age.

Lesions of the eye and ocular media:

Although diagnoses of ocular abnormalities are usually apparent from clinical examination, the VEP can be used to provide presurgical information about the integrity of the afferent visual pathways in conditions such as congenital cataracts. The VEP can also be used to assess visual acuity and guide management in conditions such as congenital glaucoma and retinal dystrophies.

Lesions of the afferent visual pathway:

In conditions such as optic nerve hypoplasia, optic nerve atrophy or glioma of the optic nerve or chiasm, the VEP can be used to assess the degree of functional visual loss and monitor disease progression and / or recovery.

Delayed visual maturation:

VEPs can be useful in infants with delayed visual maturation (DVM) to differentiate between DVM and more serious aetiologies of permanent visual impairment. Flash and pattern VEPs are usually present in DVM although they may have prolonged latencies, small amplitude or abnormal waveforms (105). The VEPs normalise as vision recovers spontaneously in these patients.

Amblyopia:

Amblyopia is subnormal visual acuity in one or both eyes despite correction of any significant refractive error. The word is used to denote a specific

developmental disorder of visual function arising from sensory stimulation deprivation. Amblyopia is usually asymptomatic and is detected only by screening programmes. Monocular pattern VEPs are sensitive for early detection of amblyopia and can be used to monitor treatment. The typical findings in amblyopia are small amplitude VEPs and absent VEPs to small check sizes in the amblyopic eye. During occlusion therapy, VEPs become equal and symmetrical as acuity equalises in the two eyes.

Cortical blindness:

Chronic cortical blindness is usually due to prenatal or perinatal events whereas acute-onset cortical blindness results from an insult to the posterior visual pathways. The pattern VEP is useful in determining the level of visual function in infants with chronic cortical blindness. In children with acute-onset cortical blindness, the flash VEP is a useful prognostic test for visual recovery (81).

Special circumstances: infants with nystagmus

In adult subjects, pattern onset and pattern reversal VEPs were compared between subjects with nystagmus and those with normal vision using checkerboard stimuli of two sizes (120’ and 60’). In the presence of nystagmus, pattern reversal VEPs were significantly smaller and of poorer quality than those obtained by pattern-onset stimuli (106). This study confirmed similar findings from other authors suggesting that pattern-reversal stimulation is not reliable in patients with horizontal nystagmus (107,108). Therefore, in the presence of nystagmus, the recommended method of pattern VEP testing is pattern onset.

1.4.5 Subject variables and the VEP

Sleep state: In young infants sleep state and eye opening state may influence the flash and flicker VEP (82,109-111). Sleeping infants have longer latency components compared to alert infants and the VEP amplitude is significantly smaller during quiet sleep compared to awake in both term and preterm infants. Documentation of sleep and eye opening state is therefore important during VEP recording. Sleep state can be defined using a behavioural scoring system assessing eye opening state, body movements, facial movements and vocalisations (112). This can be simplified to four sleep states: quiet sleep, active sleep, quiet wakefulness and active wakefulness.

Intrauterine growth restriction: Infants with IUGR were found to have smaller amplitude flash VEPs compared to normally grown controls by Stanley *et al* (113). However, all these IUGR infants had detectable VEPs of comparable waveform to normally grown control infants.

Head size: The relationship between VEP latency and both sex and head size was investigated by Gregori *et al* (2006) who recorded pattern-reversal VEPs in healthy adult subjects (114). They found the P100 latency to be shorter in female subjects with smaller head circumference although there was no difference in the subgroup of the two sexes with a comparable range of head sizes. They concluded that the difference in VEP latency between groups was attributable to head size and not to sex. Malcolm *et al* have investigated gender and OFC differences in healthy term infants undergoing pattern-reversal VEPs (91): they found shorter peak latencies in infants with smaller head circumference but also found an independent effect of gender on P1 latency, with females demonstrating shorter peak latencies than males. Accurate recording of growth parameters, including occipito-frontal circumference, and sex is therefore important when comparing VEPs.

1.4.6 Drug misuse and the VEP

Drug and alcohol misuse are associated with alterations in visual electrophysiology in adults and in animal subjects (115-118). Rat pups born to methadone-exposed mothers demonstrated delayed latency flash VEPs and reduced amplitude flicker VEPs compared to controls at the peak of the abstinence syndrome (119). There were no long term effects and all VEPs were normal 21 days after birth.

Methamphetamine misuse causes a delay in P100 peak latency of pattern VEPs in adult subjects compared to controls. It is suggested this may be due to dopamine depletion during long term drug misuse (117). Pattern VEPs in adult subjects exposed to methadone demonstrate a delay in N75 and P100 compared to normal controls (115). Proposed mechanisms for these alterations in visual sensitivity include increased turnover of dopamine in the retina and an adverse effect on neural transmission within primary visual areas of the brain. Pattern VEPs in adult subjects with previous cocaine use demonstrate a significantly delayed P100 latency, conceivably due to the vasoconstrictive affect of cocaine on the retinal, optic nerve and occipital vasculature (116). Chronic alcoholism has also been shown to have an effect on the pattern VEP with reported abnormalities including delayed P100 and abnormal VEP waveform (118,120).

Whitham *et al* (2010) have recently described pattern-reversal VEPs recorded at four months of age in infants who had been exposed to methadone *in utero* and buprenorphine *in utero* compared to control infants (121). They recruited 30 buprenorphine-maintained women, 22 methadone-maintained women and 33 non-opioid dependent controls. They found that the methadone exposed infants had significantly delayed P1 latencies in response to 48’ and 69’ check sizes compared to the buprenorphine exposed infants and controls. No neonatal testing was undertaken in this study and the results were not correlated with longer term visual outcomes.

1.5 Conclusion

Maternal drug misuse is a significant problem in pregnancy and can seriously affect the health of not only the mother, but also the fetus and newborn infant. The currently recommended treatment for pregnant opiate-dependent women is prescribed substitute methadone, but in common with other illicit drugs used in pregnancy this crosses the placenta and enters the fetal circulation.

The association of maternal drug misuse with prematurity, IUGR and NAS is well recognised and there is increasing evidence of longer term impact on infant visual and neurodevelopmental outcome. Most of the evidence regarding longer term visual outcomes in particular derives from small uncontrolled studies, with no adequately powered controlled studies published to date.

The VEP can be used to assess the integrity and maturity of the infant visual pathway. Testing is non-invasive and can be easily performed in the neonatal period. Both visual and neurodevelopmental abnormalities can be predicted by abnormal VEPs in infancy, with abnormalities including absent responses, delayed latencies and immature morphology. Drug misuse is associated with alteration of the VEP in adult humans and in animal models. To date, however, few studies have investigated the effects of maternal drug misuse upon the newborn infant VEP.

This study investigates in detail the effects of prescribed methadone and additional illicit drug use in pregnancy upon the newborn infant VEP. It aims to define abnormalities of the newborn VEP by comparison with matched infants and, by repeating VEPs in these cohorts at six months of age, to assess whether *in utero* drug exposure has a longer-term effect on visual electrophysiology. Extensive data will be collected regarding drug exposure, including history and toxicology analyses, allowing investigation of the effects of individual drugs of misuse on the VEP, both at birth and at six months of age.

The study also aims to determine visual and neurodevelopmental outcomes in later infancy in this cohort. By performing a combined clinical visual and neurodevelopmental assessment at six months of age, it describes the incidence

and scope of abnormalities and assesses how these relate to a history of NAS and the pattern of *in utero* drug exposure.

The study seeks to determine the predictive role of the VEP in the early detection of visual and neurodevelopmental abnormalities secondary to maternal drug misuse in the knowledge that, if the VEP correlates with longer term visual and/or developmental outcome, this relatively simple and inexpensive investigation could trigger earlier referral to appropriate specialists and result in improved outcome in this vulnerable group of infants.

The study also seeks to investigate the potential value of the VEP in predicting the onset and severity of NAS. This condition has significant health implications for the infant, family unit and health care resources but its pathophysiology is poorly understood. If the VEP were found to be predictive of NAS, this technique could be used to improve management of these infants by allowing earlier targeted treatment of NAS and earlier hospital discharge of infants unlikely to be affected.

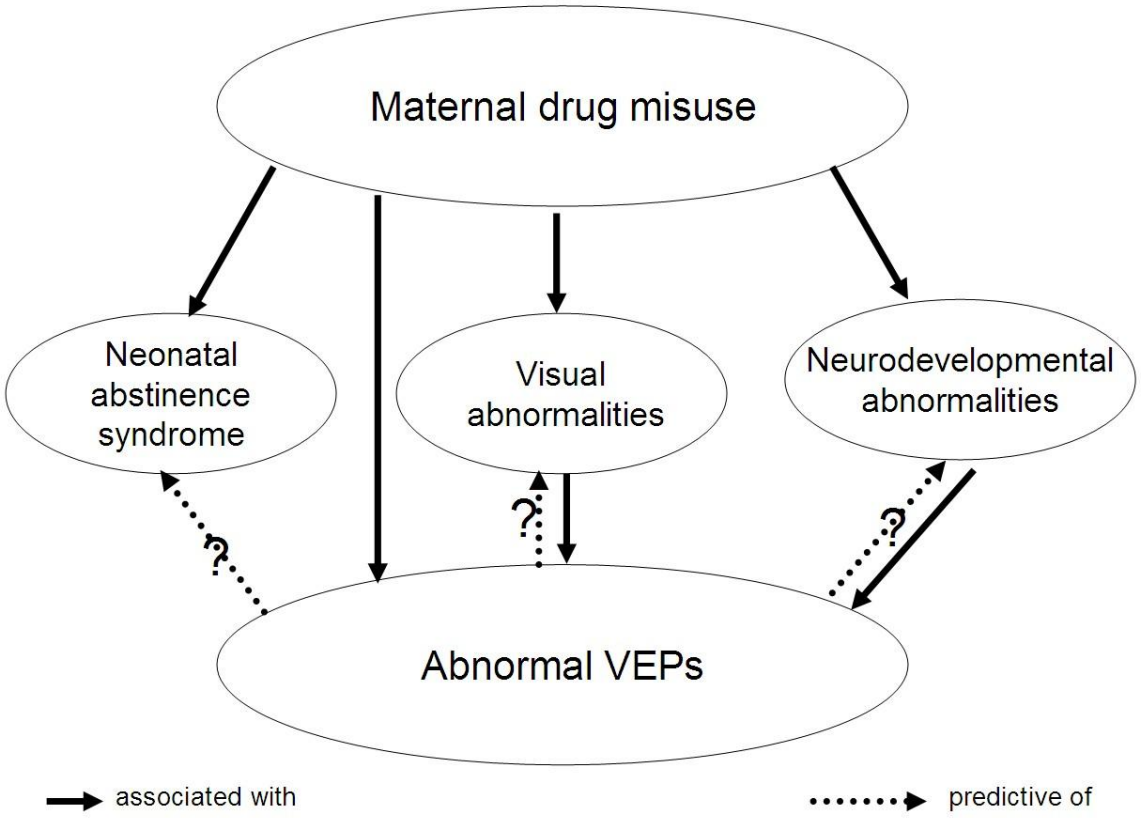


Figure 1-9 Drug misuse flowchart
Relations between maternal drug misuse, NAS, visual abnormalities, neurodevelopmental abnormalities and VEPs.

2 Chapter 2 Pilot studies

2.1 Flash Visual Evoked Potentials in newborn infants exposed to methadone *in utero*

2.1.1 Introduction

Drug misuse has been demonstrated to cause an alteration of the VEP in human adults as well as in animal studies, and maternal drug misuse has been associated with impaired infant visual development (61,62,64,115,119). A pilot study was undertaken to test the hypothesis that maternal drug misuse in pregnancy is associated with an alteration of the VEP in the neonatal period.

2.1.2 Aims

- To assess the feasibility of measuring flash VEPs in newborn infants exposed to methadone *in utero*.

- To compare flash VEPs recorded in the first few days after birth from infants exposed to methadone *in utero* and from non-maternal drug exposed control infants.

- To describe the short term maturation of VEPs in drug-exposed infants.

2.1.3 Subjects and Methods

This was a prospective case-control pilot study. Eligible infants were born at term (≥37 completed weeks gestation) to drug-misusing mothers who had been prescribed substitute methadone during pregnancy. Exclusion criteria were ocular abnormalities, other major congenital abnormalities and significant neonatal illness. Infants were recruited from the postnatal ward of the Women's Reproductive Health Service in the Princess Royal Maternity, Glasgow. Control subjects were healthy term infants born in the study hospital, using identical exclusion criteria. The multidisciplinary care package offered to drug misusing women included a postpartum stay of up to ten days to monitor for signs of NAS

and to provide parenting support. Infants were assessed for significant NAS using a modified Lipsitz score undertaken twice daily by experienced midwifery staff. Significant NAS was diagnosed if two modified Lipsitz scores at 12 hourly intervals were $\geq$5, and the infant was unusually difficult to console and/or feeding poorly. Pharmacological treatment was then commenced, with oral morphine solution as first line therapy.

Drug exposure *in utero* was determined from maternal history and infant urine toxicology. Infant bag urine samples were obtained after consent and before administration of any medication to the baby. Samples were stored at -20^{o}C until analysis in a single batch on an Abbott Architect c8200 analyser (Abbott, Abbott Park, IL, USA), using Abbott Multigent reagents (EMIT immunoassays) according to manufacturer's instructions. Assays included opiates, methadone, benzodiazepines, amphetamines, cannabinoids and cocaine metabolites.

The first VEP recording was performed within four days of birth, and a second recording was undertaken after one week if the infant remained in hospital. VEPs were recorded from the occipital scalp using three silver-silver chloride electrodes in the midline occipital (recording), midline frontal (reference) and mastoid (ground) positions. Electrode positions were determined using the standard 10:20 clinical montage (75). Scalp-electrode impedance was measured before each recording and electrodes repositioned if necessary to ensure that impedance was below 10 kohms. A hand-held integrating sphere (Colorburst®, Diagnosys LLC, Lowell, MA 01854) was presented 5 cm from the infant's eyes in the midline and delivered bright white flashes (50 cd s/m^2) at 1 Hz. All stimulation was binocular. A minimum of two averaged VEPs of 30 flashes each were collected to ensure reproducibility. Awake/sleep state and degree of eye opening were documented. VEPs were stored and subsequently assessed by two independent observers, blinded to the infant's clinical course. VEPs were classified as *typical* (predominant positivity near 200 ms, P2), *atypical* (more complex response with unusual peak latencies), *immature* (predominant negativity near 300 ms, N3), or *non-detectable* (Figure 2-1). The largest peak to trough amplitude was measured for all detectable VEPs and the total sum amplitude of all peaks and troughs was calculated. When present, peaks and troughs were labelled in order of increasing latency as P1, P2, N3, P3 and

amplitude from baseline and latency noted. Each outcome was compared between methadone-exposed and control infants.

The study was approved by Glasgow Royal Infirmary research ethics committee (REC reference: 06/S0704/5) and written informed parental consent obtained for all infants.

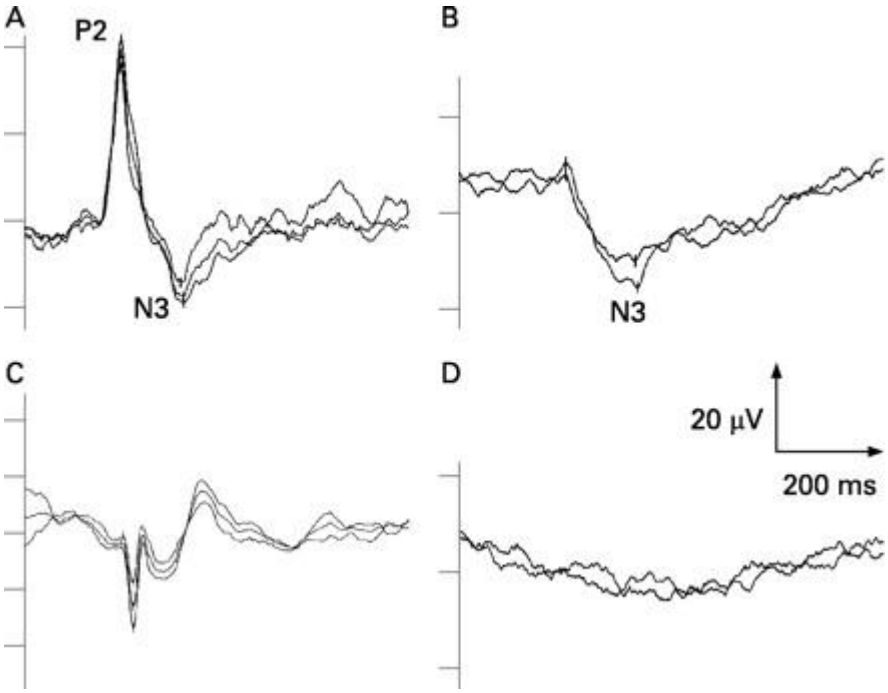


Figure 2-1 VEP classification

***A. Typical* VEP response: Predominant positivity at ~ 200ms (P2).**

***B. Immature* response: Predominant negativity at ~ 300 ms (N3).**

***C. Atypical* response: Complex response with unusual peak and trough latencies.**

***D. Non-detectable*: No reproducible VEP present.**

2.1.4 Results

Twenty-one methadone-exposed infants and 20 control infants were recruited. The characteristics of the two groups are described in Table 2-1. The maternal methadone-exposed group differed from the control group with respect to birth weight (2818 gm *vs* 3486 gm; p<0.001) and head circumference (32.9 cm *vs* 34.9 cm; p<0.001). The methadone-exposed infants were also of earlier gestation (38.6±1.4 *vs* 39.8±0.95 weeks; p=0.002).

The first VEP was recorded at a median age of 30 hours – this age did not differ significantly between the groups. VEPs were repeated after one week in 14 of the maternal methadone-exposed infants, seven of whom developed significant NAS. Eight methadone-exposed infants in total developed NAS requiring pharmacological treatment. Interpretable data were obtained in 54/55 VEP sessions; in one session the infant was too unsettled to allow a successful recording (VEP was successfully recorded two days later).

Toxicology: Eleven of the 13 infant urine samples obtained were positive for methadone. For both negative infant samples, maternal urine testing confirmed methadone use during late pregnancy. Other substances detected in infant urine included benzodiazepines (8/13), cocaine (2/13) and cannabinoids (1/13).

VEPs: At 1-4 days of age, maternal methadone-exposed infants had an abnormal distribution of VEP waveforms compared to controls infants (Chi^2 =12.0, p< 0.01), with fewer typical VEPs and more immature waveforms. VEPs were non-detectable in five cases (Figure 2-2).

Table 2-1 Pilot patient demographics

	Methadone (n=21)	*Control (n=20)*	*p value*
Gestation (weeks)	38.6 (1.4)	39.8 (0.95)	0.002
Male: Female	10:11	10:10	0.879
Birth weight (grams)	2818 (533)	3486 (535)	<0.001
OFC (cm)	32.9 (1.38)	34.9 (1.45)	<0.001

Data are means (standard deviations).

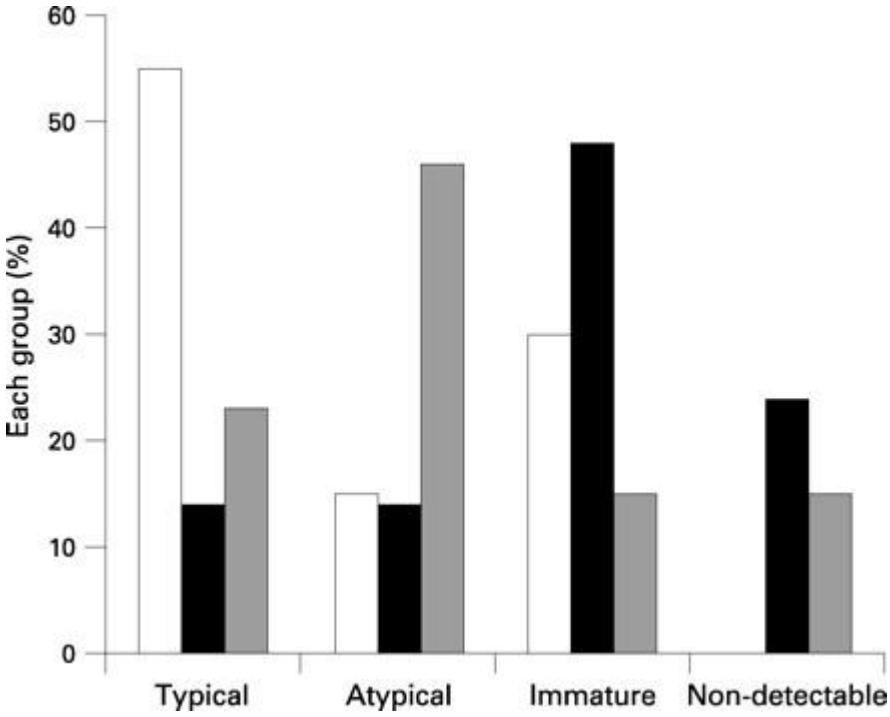


Figure 2-2 Distribution of VEP waveforms.
White: control group (n=20). Black: drug-exposed infants at a median age of one day (n=21). Grey: drug-exposed infants at a median age of eight days (n=14). Data are expressed as a percentage of each group.

Median total sum amplitude was significantly different between groups: 17μV for the methadone-exposed group and 30μV for the control group (Mann-Whitney test, 95% CI of difference 5–25μV, p=0.002). All measured peaks and troughs for methadone-exposed infants tended to have longer mean implicit times than controls and the difference was significant for P2: P1, 132ms versus 117ms (p=0.4); P2, 240ms versus 189ms (p=0.004); N3, 315ms versus 302ms (p=0.6); P3, 394ms versus 334ms (p=0.2). Amplitudes of P1, P2 and N3 were smaller for methadone-exposed infants than controls: P1, 3μV versus 5μV (p=0.02); P2, 14μV versus 18μV (p=0.009); N3, 10μV versus 16μV (p=0.045). P3 amplitude did not differ between groups.

Sleep and eyelid closure had no significant effect on the amplitudes of these bright flash VEPs but P2 was prolonged in sleep (215±28 ms) compared with P2 of awake infants (194±16 ms; p=0.03).

After one week VEPs in the methadone-exposed infants had an increased proportion of typical, and fewer non-detectable VEPs (Figure 2-2) but amplitude remained low (median 11.3; range 0-21). There were no significant differences in any of the measured VEP parameters between the seven infants who did and the seven infants who did not develop NAS.

2.1.5 Conclusions

These pilot data showed significant differences in the neonatal flash VEP of infants exposed to methadone *in utero* compared with non-drug-exposed control infants. VEPs in drug-exposed infants were more likely to be absent and, if present, to show delayed peak and trough latencies, smaller amplitudes and immature waveform.

These pilot data suggested a need for a further study of VEPs in infants exposed to drug misuse *in utero*. Questions raised included whether the VEP could be of use in predicting the onset of NAS and/or predicting visual and neurodevelopmental abnormalities secondary to maternal drug misuse. Longer term follow up of a larger number of maternal drug-exposed infants, along with comprehensive toxicology was required to address these questions, and to

determine any relationship between the neonatal VEP and the pattern of maternal drug misuse in pregnancy.

2.2 Flicker Visual Evoked Potentials in healthy term newborn infants

2.2.1 Introduction

An alternative method of assessing visual pathway function in the newborn period uses steady state luminance stimuli, otherwise described as the flicker VEP. Infants show maturation of the optimal stimulus frequency in the first year of life, reaching typical adult values of 12 to 15Hz by approximately 15 months of age. An optimal stimulus frequency of 4-5Hz has been reported in infants during the first few months of life (86,88) but very few studies have reported flicker VEPs in newborn infants.

2.2.2 Aims

- To describe flicker VEPs in a cohort of healthy term newborn infants.

- To determine optimal flicker frequencies and luminance, to guide the testing protocol for subsequent studies of maternal drug-exposed infants.

2.2.3 Subjects and Methods

Subjects:

This was a prospective observational study carried out at the PRM, Glasgow. All healthy, term (≥37 completed weeks gestation) infants born in the study hospital were eligible for inclusion. Exclusion criteria were significant neonatal illness, congenital ocular abnormality or a history of *in utero* exposure to drug misuse. All infants were ≤48 hrs of age at the time of testing. Informed parental consent was obtained for all participants and Research Ethics Committee approval was obtained prior to study commencement.

Recording:

VEPs were recorded in a dimly illuminated room with the infant placed supine in a cot. Recording sessions were timed to suit the infant's feeding schedule in order to maximise co-operation. VEPs were recorded using three silver-silver chloride electrodes in the midline occipital (recording), midline frontal (reference) and mastoid (ground) positions. Electrode positions were determined using the standard 10:20 clinical montage. Scalp-electrode impedance was measured before each recording and was below 5 kohms. Awake/sleep state and degrees of eye opening were documented. The VEP signals were amplified and band-pass filtered (0.6-100Hz).

Stimuli:

A hand held LED-based stimulator (Colorburst®, Diagnosys LLC, Lowell, MA 01854) was used to present the flickering light 5cm from the infant's eyes in the midline. Frequencies of 2.9 Hz, 4.64 Hz, 7.3 Hz, 12.7 Hz, 18.55 Hz and 38.1 Hz were presented at two different luminances (80 cds/m^2 and 500 cds/m^2), subsequently referred to as dim and bright flicker respectively. All stimulation was binocular. The stimulus was a white square waveform. A sample frequency of 1000Hz was used and the time window varied according to the frequency under investigation (4096ms for 2.9Hz and 4.64 Hz, 2048ms for 7.3 Hz, 1024ms for 12.7 Hz, 18.5 Hz and 38.1 Hz).

Data processing and analysis:

Each averaged VEP was subjected to resampling and Fourier analysis (84). Noise was estimated as the average of the two neighbouring spectral lines (one below and one above the response frequency). A significant response was defined as a signal to noise ratio (SNR) of > 2.82 which corresponds to a p value < 0.05 (85). A significant response at each flicker frequency was sought for both the fundamental response (F1) and its first harmonic (F2) (Figure 2-3, Figure 2-4). The optimal stimulus frequency was described for each recording, defined as the frequency that elicited the highest amplitude response at F1.

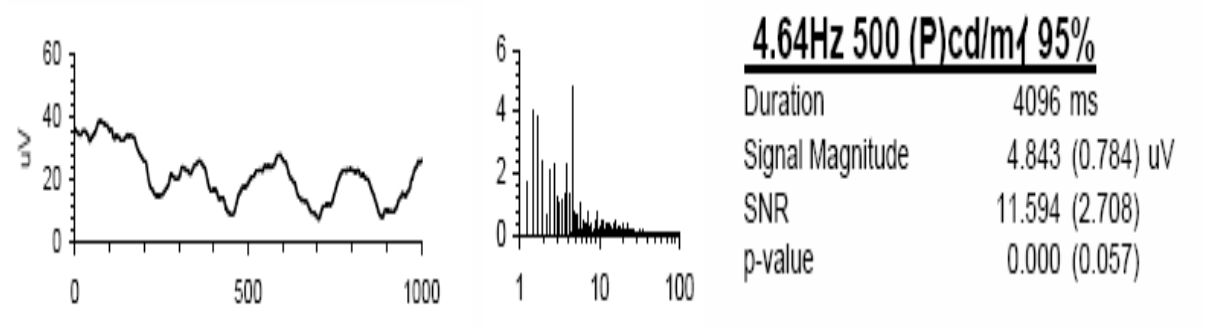


Figure 2-3 Flicker F1 response
Flicker VEP recorded on day 1 of life using a bright flicker light stimulus with a frequency of 4.6Hz. A significant response is shown at F1 (signal magnitude 4.8, Signal to Noise Ratio 11.6, p < 0.001) as demonstrated by the spike at 4.6Hz. No response is seen at F2.

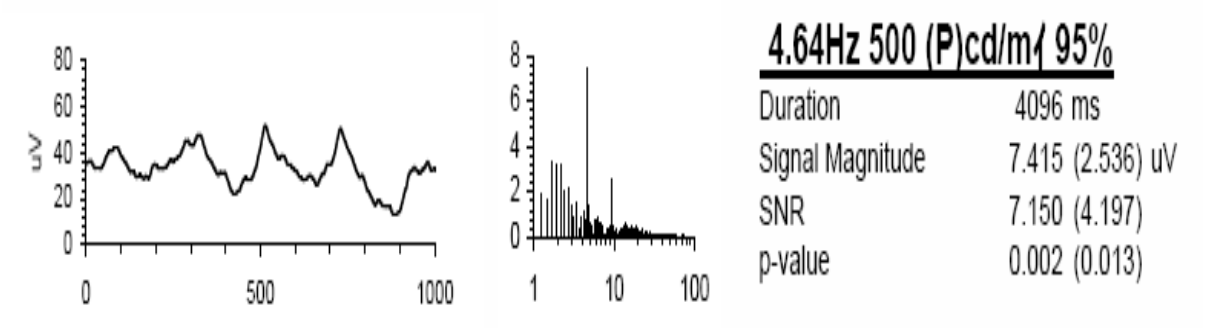


Figure 2-4 Flicker F1 and F2 response
Flicker VEP recorded on day 2 of life using a bright flicker light stimulus at a frequency of 4.6Hz. A significant fundamental response is seen at F1 (signal magnitude 7.4, Signal to Noise Ratio 7.15, p = 0.002) and a significant harmonic response is seen at F2 (signal magnitude 2.5, Signal to Noise Ratio 4.1, p = 0.013).

2.2.4 Results

Flicker VEP recordings were obtained in 34 healthy term newborn infants within the first two days of life. Gestation ranged from 37-41 weeks (median 39.5). The mean birth weight was 3468 grams (standard deviation 531 grams). Flash VEPs were recordable from all infants.

Unlike the flash VEP, not all infants demonstrated a flicker response. The proportions of infants with flicker responses at the dim and bright light stimuli are shown in Figure 2-5. The bright light stimulus more often produced a VEP response than the dim light stimulus. This was statistically significant at 4.6 Hz (66% response with bright stimulus *vs* 23% response with dim stimulus; Chi ^{2} = 13, p<0.001), 12 Hz (23% response with bright stimulus *vs* 9% response with dim stimulus; Chi ^{2}= 2.7, p=0.090) and 18 Hz (31% response with bright stimulus *vs* 6% response with dim stimulus; Chi ^{2}= 7.7, p=0.004). Overall, the greatest number of responses was obtained using the bright 4.6 Hz stimulus (66% response rate). Only a minority of infants demonstrated a response above 18 Hz.

Flicker F1 amplitude data were not normally distributed using Anderson-Darling tests for normality: data were therefore described as medians and inter-quartile ranges and statistical analysis done with Mann-Whitney tests. The bright light stimulus consistently produced larger amplitude F1 flicker responses at all frequencies. This was statistically significant at 4.64 Hz, 7.3 Hz and 18.5 Hz (Table 2-2) (Figure 2-6). The largest amplitude responses were produced using the 4.64 Hz bright light stimulus and the magnitude of response declined with increasing stimulus frequency.

An association was investigated between the flash VEP amplitude and flicker VEP amplitude. Both flash P2 amplitude data and flicker F1 amplitude data were of skewed distribution and were therefore logarithm transformed to a normal distribution for investigation of any linear relationship between the variables. A fitted-line scatter plot and regression analysis were undertaken (Figure 2-7). There was a positive linear correlation between flash and flicker VEP amplitude: R^2 17.8%, p=0.023.

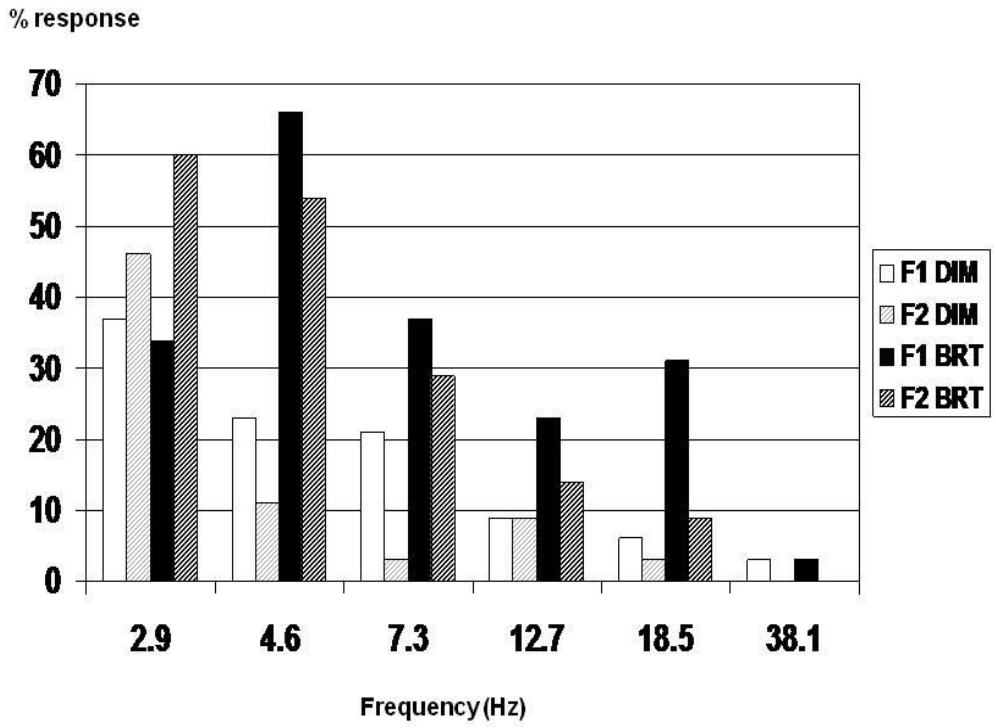


Figure 2-5 Flicker VEP response
The graph demonstrates the proportion of infants with a flicker VEP response at frequencies ranging from 2.9 Hz to 38.1 Hz. Responses to dim and bright light stimuli are shown and both the fundamental response (F1) and the first harmonic are shown (F2). The greatest proportion of responses was obtained using the 4.6 Hz bright flicker stimulus.

Table 2-2 Flicker F1 VEP amplitudes

Freq	Dim stimulus	Bright stimulus	p-value
2.9 Hz	2.79 (1.50-5.03)	2.98 (1.85-4.66)	0.946 (-0.87,0.86)
4.64 Hz	1.09 (0.66-1.75)	3.41 (2.60-4.59)	**<0.001** (-2.82,-1.68)
7.3 Hz	0.85 (0.46-1.13)	1.16 (0.61-1.56)	**0.048** (-0.53,0.007)
12.7 Hz	0.47 (0.33-0.71)	0.58 (0.42-0.95)	0.057 (-0.30,0.003)
18.5 Hz	0.25 (0.16-0.34)	0.46 (0.31-0.71)	**<0.001** (-0.32,-0.10)
38.1 Hz	0.08 (0.04-0.12)	0.09 (0.05-0.15)	0.300 (-0.05,0.01)

Data are medians (inter-quartile ranges). Statistical analysis was done using Mann-Whitney tests. The 95% confidence interval for difference is given in brackets after the p-value. The bright flicker stimulus produced larger amplitude F1 responses at all frequencies which was statistically significant at 4.64 Hz, 7.3 Hz and 18.5 Hz. The optimal stimulus frequency was 4.64 Hz.

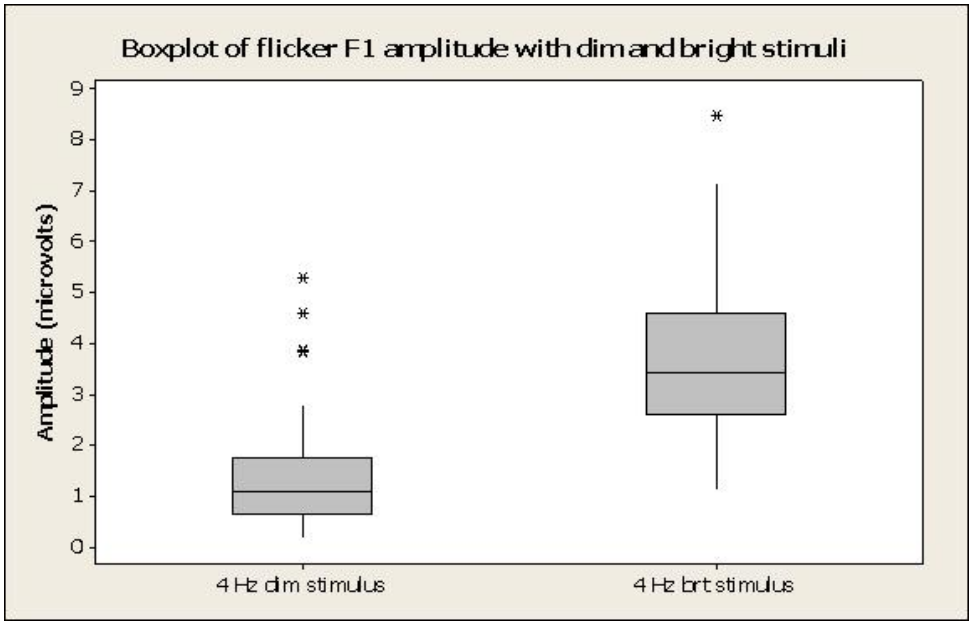


Figure 2-6 Boxplot of flicker amplitude
The bright flickering 4.64 Hz stimulus produced larger amplitude responses compared to the dim stimulus; Mann-Whitney test: p<0.001.

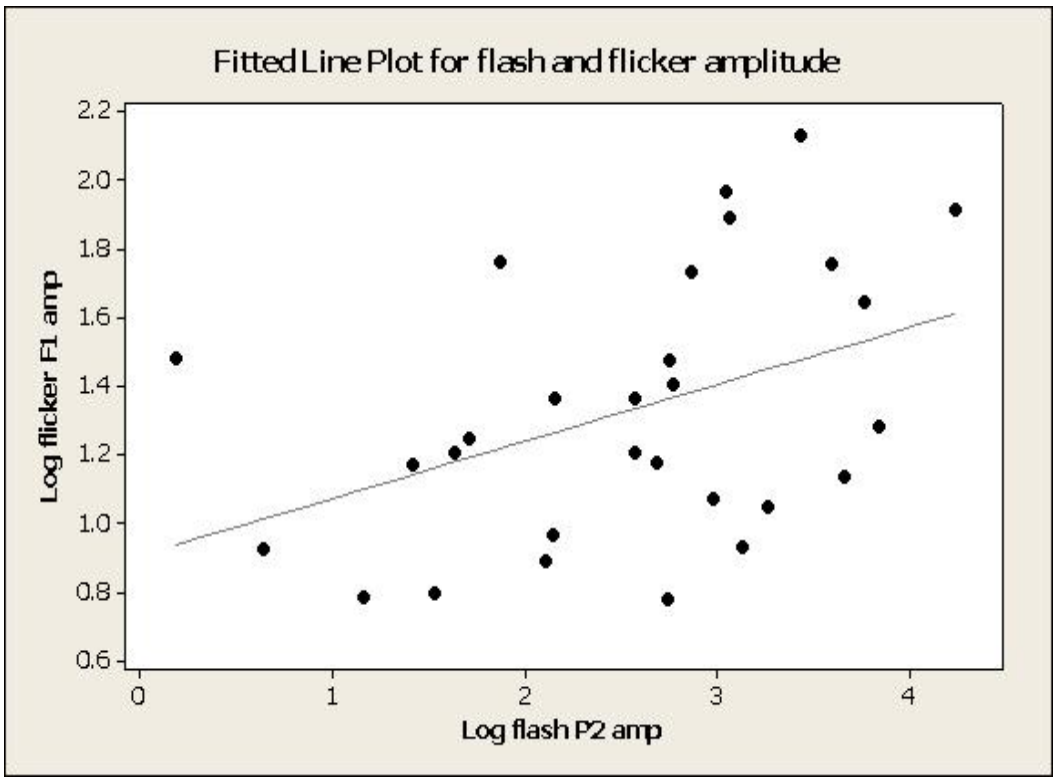


Figure 2-7 Scatterplot of flash and flicker amplitude
There was a positive linear association between the flash and flicker VEP amplitude data (R^2 17.8%, p=0.023).

2.2.5 Conclusion

This study provides normative data for flicker VEPs in healthy term newborn infants and allows comparison between the flicker stimuli of two different luminances.

In common with other electrophysiological tests of visual function in the newborn, a bright flicker stimulus more reliably produced significant responses than a dim stimulus. The optimal stimulus flicker frequency demonstrated in this study was 4.6Hz which is in keeping with previous reports from older infants (87,88). Very few infants demonstrated responses above 18Hz. This study suggests that optimal flicker VEPs are achieved using a bright (500cds/m^2) flickering light stimulus at approximately 5Hz in the newborn infant. Modification of future protocols for testing in newborn infants should include an additional testing frequency at approximately 5 Hz and removal of the 38Hz frequency.

2.3 Visual Evoked Potentials in preterm infants

2.3.1 Introduction

Immature flash VEPs, similar to those of moderately preterm infants, have been described in newborn infants exposed to methadone *in utero* in pilot study 1(122). Since flicker VEP recording is a more objective method applicable in the newborn period there is a requirement for established normative values for flicker VEPs in healthy moderately preterm infants.

2.3.2 Aims

To describe flash and flicker VEPs in a group of moderately preterm infants.

2.3.3 Subjects and Methods

This was a prospective observational study. Inclusion criteria were moderately preterm infants (33 – 35+6 weeks' gestation) born at the PRM who were clinically stable and appropriately grown (birth weight >10th centile and <90th centile). Exclusion criteria were congenital ocular abnormality, other significant congenital abnormality, significant neonatal illness and/or a history of maternal drug misuse in pregnancy. To provide a comparative group 14 healthy term infants were matched to the preterm infants for sex, DEPCAT score (123) and maternal smoking status.

2.3.3.1 Recording

VEPs were recorded within 12 to 72 hours of birth. VEPs were recorded in a dimly illuminated room with the infant placed supine in their cot or incubator. Each recording session was timed according to the infant's feeds, to maximise co-operation. VEPs were recorded using three silver-silver chloride electrodes in the midline occipital (recording), midline frontal (reference) and mastoid (ground) positions. Scalp-electrode impedance was measured before each recording and was below 5 kohms. Awake/sleep state and degrees of eye opening were documented. The VEP signals were amplified and band-pass filtered (0.6-100Hz).

2.3.3.2 Stimuli

A hand held LED-based stimulator (Colorburst®, Diagnosys LLC, Lowell, MA 01854) was used to present the flash and flicker light 5cm from the infant's eyes in the midline. Bright (50 cds/m^2) and dim (5 cds/m^2) white flashes were delivered at 1 Hz and a minimum of two averaged VEPs of 30 flashes each were collected to ensure reproducibility. A bright flickering light stimulus was then presented at frequencies of 4.64 Hz, 5.86 Hz, 7.32 Hz, 12.7 Hz and 18.55 Hz. These flicker frequencies were modified from those used in the pilot study of term newborn infants: an additional frequency was added at 5.86 Hz as the pilot study had demonstrated an optimal response at 4.6 Hz, and the 38 Hz frequency was removed as few term infants had demonstrated a response at this level. Two different flicker stimuli were used at each frequency – a pulse wave and a sine wave. All stimulation was binocular. A sample frequency of 1000Hz was used and the time window varied according to the frequency under investigation (4096ms for 4.64 Hz, 2048ms for 5.86 Hz and 7.32 Hz, 1024ms for 12.7 Hz and 18.55 Hz).

2.3.3.3 Data processing and analysis

Flash VEPs

VEPs were classified as present or absent. When present, peaks and troughs were labelled in order of increasing latency as P1, P2, N3, P3 and amplitude from baseline and latency noted. Total sum amplitude of all peaks and troughs was calculated. The morphology of the preterm waveform was described relative to term controls infants.

Flicker VEPs

Each VEP was subjected to resampling and Fourier analysis (85). Noise was estimated as the average of the two neighbouring spectral lines (one below and one above the response frequency) and a significant response was defined as a signal to noise ratio (SNR) of >2.82 which corresponds to a p value <0.05 (85). A significant response at each flicker frequency was sought for both the fundamental response (F1) and the first harmonic (F2). The optimal stimulus

frequency was also described, defined as the frequency which elicited the highest amplitude response at F1.

The study was approved by the Glasgow Royal Infirmary research ethics committee (REC reference number 09/S0704/2) and informed parental consent was obtained for all participants.

2.3.4 Results

2.3.4.1 Demographics

Fourteen preterm infants and 14 term infants matched for sex, DEPCAT score and smoking status were recruited to the study. The median gestation of the preterm group was 35 weeks (IQR 34-35) and of the term group was 39 weeks (IQR 38-41). Mean birth weight and head circumference of the preterm infants were significantly lower than that of the term infants (mean birth weight 2219 grams *vs* 3219 grams, $p<0.001$; mean OFC 31.4 cm *vs* 35.1 cm, $p<0.001$).

2.3.4.2 Flash VEPs

Significantly fewer preterm infants had a VEP response with the dim light stimulus compared to term infants. When a response was present it was of reduced amplitude and delayed P2 latency (Table 2-3).

All preterm and term infants had a VEP response to the bright light stimulus. Significantly fewer preterm infants had a P1 response compared to term infants. Mean P2 latency with the bright light stimulus was again delayed in the preterm group; however this was not statistically significant. Total VEP amplitude was similarly reduced with the bright light stimulus (Table 2-3, Figure 2-8).

Table 2-3 Flash VEPs in preterm and term infants

	DIM FLASH			BRIGHT FLASH		
	Preterm	*Term*	*p-value*	*Preterm*	*Term*	*p-value*
VEP present	50%	100%	**0.006**	100%	100%	1.000
P1 present	0%	7%	1.000	0%	50%	**0.006**
P2 present	29%	93%	**0.001**	100%	100%	1.000
P2 latency (ms)	260 (13.3)	218 (32.7)	**0.028**	250 (44.6)	224 (37.1)	0.104
Total amp (μV)	1.15 (0-7.8)	25.45 (9.7-43.3)	**<0.001**	22.6 (17.4-31.2)	48.6 (27.9-69.7)	**0.006**

Data are percentage response (%), mean (standard deviation) for P2 latency and median (inter-quartile range) for amplitude.

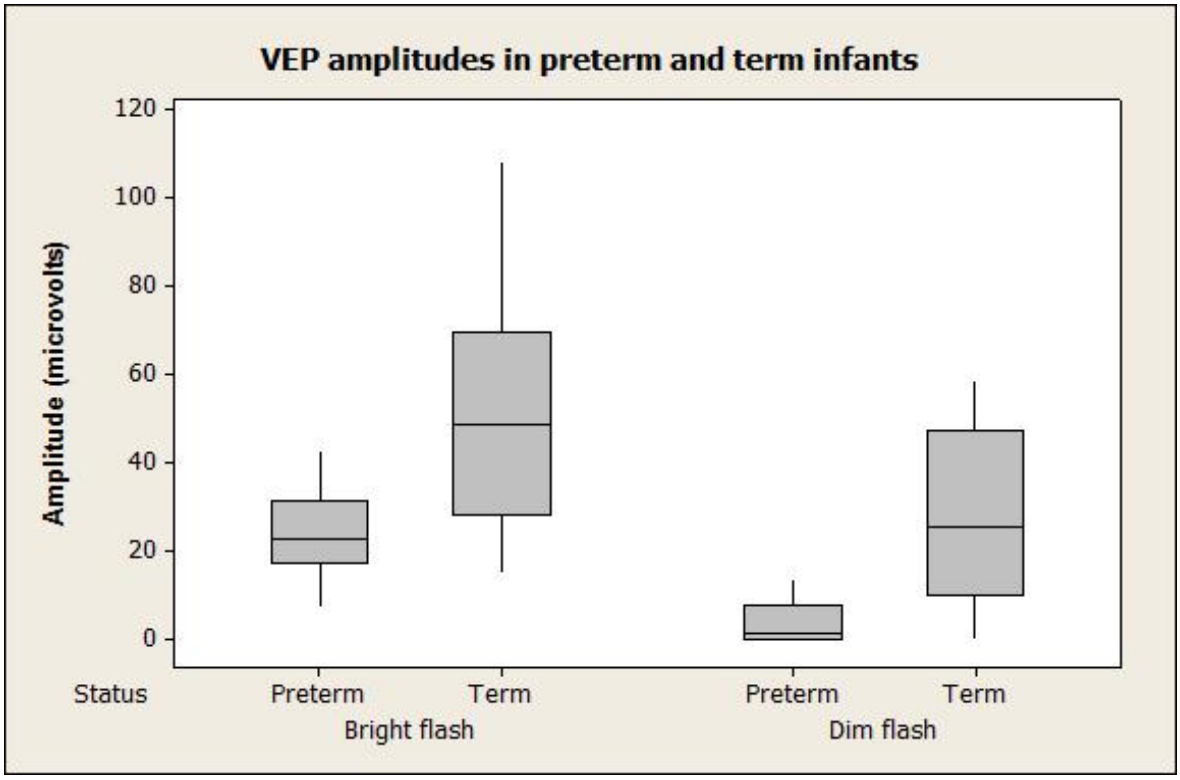


Figure 2-8 Boxplot of flash VEP amplitude in preterm infants.
Preterm infants had significantly smaller amplitude responses compared to term infants with both the bright flash stimulus (p=0.006) and dim flash stimulus (p<0.001).

The flash preterm infant VEP waveforms were also used to better define an immature VEP morphology for future studies. On review of the waveforms it was found that no preterm VEPs had P1 components and all had P2 and N3 components. In 13/14 preterm waveforms, the amplitude of N3 was greater than three times the amplitude of P2 (Figure 2-9).

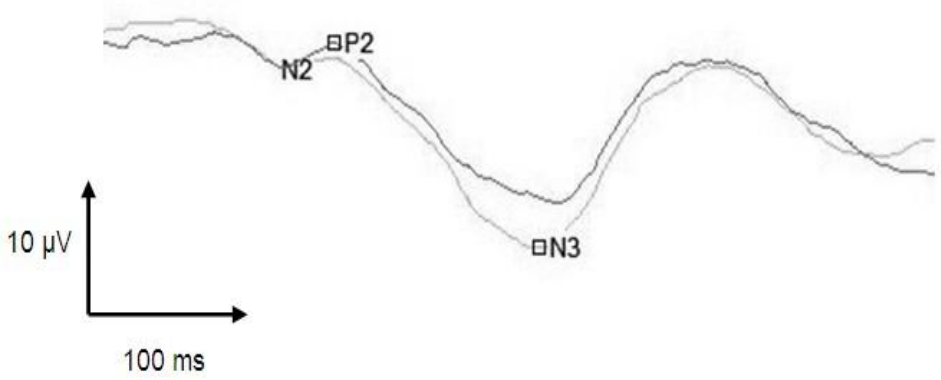


Figure 2-9 Preterm VEP waveform
A bright flash VEP waveform recorded from an infant born at 34 weeks gestation on day 2 of life. No P1 component was present. The waveform was a predominant negativity with N3 amplitude greater than three times P2 amplitude.

2.3.4.3 Flicker VEPs

Twelve preterm infants underwent flicker VEP analysis (two sets of data were lost due to a computer system failure). Results were compared to those of 14 term infants matched as described above. Similar to term infants, preterm infants had an optimal flicker response at 4.6 Hz. On statistical analysis the preterm group had a greater proportion of responses at 5.86Hz compared to controls but had fewer responses at 18.55Hz (Table 2-4). However, using a Bonferonni correction to account for the number of statistical tests performed, these differences were not significant.

Flicker F1 amplitudes were compared between the 12 preterm infants and the 14 matched term infants. There were few differences in amplitude between groups (Table 2-5). At 18.55 Hz the term infants had larger amplitude flicker F1 responses, but after using a Bonferroni correction for the number of statistical test performed this was no longer significant and may have been due to chance.

Table 2-4 Flicker responses in term and preterm infants

	Pulse wave			Sine wave		
Freq (Hz)	***Preterm***	***Term***	***p-value***	***Preterm***	***Term***	***p-value***
4.64	83%	64%	0.391	92%	79%	0.598
5.86	75%	29%	0.047	50%	79%	1.000
7.32	25%	43%	0.429	50%	57%	1.000
12.7	17%	21%	1.000	17%	14%	1.000
18.55	0%	36%	0.042	0%	14%	0.483

Statistical analysis was undertaken to investigate the flicker VEP response between preterm and term infants. Using a Bonferroni correction to account for the number of statistical tests performed, a p-value of <0.005 was considered significant.

Table 2-5 Flicker amplitude in term and preterm infants

	Pulse wave			Sine wave		
Freq (Hz)	***Preterm***	***Term***	***p-value***	***Preterm***	***Term***	***p-value***
4.64	2.74	2.77	0.777	3.67	1.96	0.095
5.86	1.51	0.89	0.269	1.44	1.88	0.857
7.32	0.94	1.43	0.129	1.36	1.67	0.341
12.7	0.78	0.59	0.487	0.84	0.85	0.699
18.55	0.42	0.61	0.033	0.49	0.47	0.738

Data are median values. Mann-Whitney tests were used for comparisons between groups.

2.3.5 Conclusion

Preterm infants had fewer flash VEP responses compared to term infants and in particular fewer P1 responses. The total VEP amplitude was reduced and P2 latency delayed. A reasonable definition for the preterm infant flash VEP waveform was: no P1 component present, predominant N3 waveform with N3 amplitude greater than three times the P2 amplitude. Preterm infants demonstrated similar flicker responses to term infants with 4 Hz to 18 Hz stimuli. However, the numbers studied may have been too small to detect significant differences between groups. Optimal stimulus frequency for both groups was 4.64 Hz.

2.4 Ocular and electrophysiology abnormalities in children exposed to methadone *in utero*

2.4.1 Introduction

There is increasing evidence to suggest that *in utero* opiate and benzodiazepine exposure has an adverse effect on infant visual development (61,64). Within the local ophthalmology and visual electrophysiology service, it was recognised that an increasing number of children were being referred with a history of maternal methadone use (personal communication – Drs R Hamilton and J MacKinnon).

2.4.2 Aims

- To describe the combined ophthalmology and visual electrophysiology findings in infants and children who had been exposed to methadone and other drugs of misuse *in utero* and referred to a regional visual electrophysiology service.

- To use this information to inform the testing protocol for the follow-up phase of the study.

2.4.3 Subjects and Methods

This was a retrospective descriptive case series of children referred to paediatric ophthalmology services because of concerns regarding visual function and who were known to have been exposed *in utero* to methadone. Ophthalmic and orthoptic examination included visual acuity, cover tests, ocular motility, cycloplegic refraction and dilated fundoscopy. Visual acuity was assessed using age-appropriate tests; some subjects were unable to cooperate with behavioural acuity tests and step VEP acuity assessment was used instead. Delayed visual maturation was a retrospective diagnosis in babies with visual behaviour which was poorer than expected for their postnatal age but which improved by six months of age. Structured history taking was used to seek evidence of cerebral visual impairment (CVI) in older children (72).

Following ophthalmology and orthoptic review, all infants were assessed in a specialist visual electrophysiology clinic where appropriate investigations were undertaken depending on the age of the child and presenting symptoms. VEPs and electroretinograms (ERGs) were recorded according to international standards, but with methods modified to suit the age of the child (75). Pattern-reversal VEPs to a black-and-white checkerboard at 100% contrast, subtending 30°×24° and reversing at 1.1Hz were recorded. Pattern-onset VEPs were recorded to a black-and-white checkerboard at 100% contrast, interleaved with an isoluminant grey screen, subtending 30°×24° and reversing at 1.1Hz. Flash VEPs were recorded to a hand-held diffuse flash with a time-integrated luminance of 11.7 cds /m^2. In order to estimate acuity, step VEPs were recorded to black-and-white reversing checks using real-time analysis and a successive approximation algorithm to find spatial thresholds (124).

Paediatric and neonatal case notes were reviewed, and details obtained regarding maternal antenatal urine toxicology when available. Subjects were excluded from the case series if they had been born before 32 weeks gestation or had another diagnosis which could potentially account for their visual abnormalities such as fetal alcohol syndrome or birth asphyxia.

2.4.4 Results

Twenty children underwent comprehensive ophthalmology and visual electrophysiology assessment. All children had been exposed to prescribed substitute methadone *in utero* and a majority had also been exposed to illicit drugs *in utero*, most commonly benzodiazepines (11/20, 55%) and heroin (8/20, 40%). Drug exposure as determined from case notes and maternal urine toxicology is shown in Table 2-6. Twelve infants (60%) had received pharmacological treatment for NAS.

Table 2-6 Drug exposure and systemic findings

Patient number	Gestation (wks)	MDN	Heroin	BDZ	Other drugs	Alcohol	Treatment for NAS
1	38	✓					
2	32	✓				✓	
3	39	✓		✓			
4	40	✓		✓	Cannabis		
5	38	✓					✓
6	39	✓	✓				✓
7	40	✓	✓	✓		✓	✓
8	38	✓			Cocaine		✓
9	36	✓	✓	✓	Cocaine		✓
10	34	✓		✓	Cannabis		✓
11	40	✓		✓	Cannabis		✓
12	35	✓	✓	✓	Cannabis		✓
13	40	✓	✓				✓
14	38	✓				✓	
15	36	✓		✓			✓
16	38	✓			Antidepressant		
17	41	✓	✓	✓			✓
18	40	✓	✓	✓	Cannabis		✓
19	37	✓					
20	39	✓	✓	✓	DF118		

MDN: methadone, BDZ: benzodiazepines, NAS: neonatal abstinence syndrome, DF118: dihydrocodeine.

Ocular and electrophysiology findings are shown in Table 2-7. The most common abnormalities demonstrated were reduced visual acuity (19/20, 95%), nystagmus (14/20, 70%), delayed visual maturation (10/20, 50%), strabismus (6/20, 30%) and refractive errors (6/20, 30%).

Significantly more infants with a history of treated NAS developed nystagmus than those without NAS: 11/12 (92%) versus 3/8 (38%); Fisher's exact test, p=0.017. Nystagmus was horizontal in nature with the majority having a pendular wave-form although one patient exhibited a jerk type pattern. The observed characteristics of the nystagmus varied in being manifest, latent, or manifest with a latent component. Interestingly, patient 13 initially had manifest latent nystagmus which developed with time into latent nystagmus alone. Fundal examination was abnormal in two cases – one case had bilateral abnormal blood vessels crossing the macula which showed regression at five months of age, and the other had slight pallor of both optic discs.

Twelve patients (60%) had abnormal visual electrophysiology. One of these (patient 11) had a reduced amplitude cone and flicker ERG, but normal fundus and normal pattern-onset VEP to 60' checks. All other ERGs recorded were normal (n=13). Flash VEPs were recorded from 11 patients and were normal in eight (73%) and delayed in three cases (patients 5, 10 and 12). Pattern-reversal VEPs were recorded from six patients and were normal in two cases but delayed or absent in four cases (patients 5, 9, 17 and 20). Pattern-onset VEPs were recorded in six cases and were normal in five but delayed in one (patient 5). Eleven subjects had their visual acuity estimated using the step VEP; in nine cases (82%), acuity was abnormal for age. Step VEP acuity estimates agreed with contemporary behavioural acuity assessments in all of the nine cases where both were available.

Five children (25%) had significant neurodevelopmental problems (developmental delay in four, cerebral palsy in one), three of whom (patients 2, 13 and 19) had CVI and one of whom (patient 12) had a delayed flash VEP. Cerebral visual impairment (CVI), causing functional visual processing problems including dorsal and ventral stream abnormalities, was screened for in the older children by structured history taking and was found in five cases (25%).

Table 2-7 Ocular and electrophysiology findings

Patient	DVM	Nystagmus	Strabismus	Refractive error	CVI	Flash VEP	P-R VEP	P-O VEP	VEP acuity
1	✓		XT			Normal			Raised
2	✓				✓		Normal		Raised
3		✓				Normal			
4	✓							Normal	Raised
5		✓				Delayed	Abnormal	Delayed	
6	✓					Normal			Normal
7		✓	ET					Normal	
8		✓				Normal			Raised
9	✓	✓		HA		Normal	Absent		Raised
10		✓				Delayed		Normal	Raised
11		✓						Normal	
12	✓	✓	XT	MA (rt eye)		Delayed			Raised
13	✓	✓	MT		✓		Normal		
14	✓		XT	H					Raised
15	✓	✓			✓	Normal			
16		✓		HA		Normal			Raised
17	✓	✓					Absent	Normal	
18		✓			✓				
19		✓	ET	HA	✓	Normal			
20				A			Delayed		Raised

DVM: delayed visual maturation, ET: esotropia, XT: exotropia, MT: microtropia. For refraction M: myopia, H: hypermetropia, A: astigmatism. CVI: cerebral visual impairment, VEP: visual evoked potential, P-O: pattern onset, P-R: pattern reversal.

2.4.5 Conclusion

Ocular abnormalities detected in infants and children exposed to methadone and other substances of misuse *in utero* included reduced visual acuity, nystagmus, delayed visual maturation, refractive errors and strabismus (125). There was also a high incidence of VEP abnormalities. Infants who have received treatment for NAS may be at particular risk of visual abnormalities, especially nystagmus.

Visual assessment of infants exposed to drug misuse *in utero* should include an assessment of visual acuity, observation for nystagmus, corneal reflexes to assess for strabismus and assessment for refractive errors; as well as measurement of VEPs. Due to the high incidence of nystagmus in this cohort, the preferred method of pattern VEP testing should be pattern-onset (106).

3 Chapter 3 Methods

Maternal drug misuse can seriously affect the health of the fetus and newborn infant. The association of maternal drug misuse with prematurity, IUGR and NAS is well recognised, and there is growing concern about infant visual and developmental outcome. Drug misuse is associated with changes in the visual system as measured by VEPs in adults and in animal models. Since visual abnormalities and neurodevelopmental abnormalities can be predicted by abnormal VEPs in infancy, it is postulated that the VEP may be a valuable tool in the detection of adverse effects of maternal drug misuse upon the infant.

Pilot work demonstrated the feasibility of recording VEPs in the neonatal period and showed abnormal VEPs in infants exposed to drug misuse *in utero* compared to unmatched controls (Chapter 2.1). Further pilot work described the scope of clinical visual abnormalities in a selected group of infants and children exposed to methadone *in utero* (Chapter 2.4). Further study was required to validate and investigate these pilot data and would require recruitment of a large number of methadone exposed infants, along with comprehensive toxicology collection and longer term follow up.

3.1 Objectives

There were two parts to the study:

Part 1: Neonatal visual evoked potentials

In the first part of the study, neonatal VEPs were measured in infants who had been exposed *in utero* to methadone and other drugs of misuse. These VEPs were compared to VEPs from matched non-maternal drug-exposed infants. Associations were sought between neonatal VEPs and the pattern of *in utero* drug exposure. Investigations were also undertaken to determine whether neonatal VEPs were predictive of the development of NAS.

Part 2: Early visual and neurological development

In the second part of the study the same cohort of maternal drug-exposed and comparison infants was followed-up at six months of age with clinical visual and electrophysiology assessment as well as assessment of overall neurodevelopmental progress. An association was sought between the neonatal VEP and both visual and developmental outcome at six months of age.

3.2 Hypothesis and aims

Hypothesis (1):

The neonatal VEP is altered in infants exposed *in utero* to methadone compared to non-exposed infants.

Aims (1):

To describe neonatal VEPs in infants exposed *in utero* to methadone.

To compare these data with data obtained from non-maternal drug-exposed infants.

Hypothesis (2):

The neonatal VEP can be used to predict which infants will develop NAS.

Aim (2):

To assess whether the neonatal VEP is predictive of NAS.

Hypothesis (3):

Clinical visual abnormalities are more common in infants exposed *in utero* to methadone than non-exposed infants.

Aims (3):

To compare visual development at six months of age in infants exposed *in utero* to methadone with that of non-maternal drug-exposed infants.

To define the incidence of visual abnormalities in infants exposed *in utero* to methadone compared to non-exposed infants.

Hypothesis (4):

Developmental abnormalities are more common in infants exposed *in utero* to methadone than non-exposed infants.

Aim (4):

To compare developmental outcomes at six months of age in infants exposed *in utero* to methadone with that of non-exposed infants.

Hypothesis (5):

The neonatal VEP can be used to predict which infants will develop visual and/or developmental abnormalities.

Aim (5):

To assess if visual and/or developmental outcome at six months of age correlates with the neonatal VEP.

Hypothesis (6):

At six months of age the VEPs differ between infants who had been exposed to methadone *in utero* and non-exposed infants.

Aim (6):

To describe and compare VEPs in infants who had been exposed to methadone *in utero* and non-exposed infants at six months of age.

3.3 Subjects and setting

The study was conducted at the Princess Royal Maternity (PRM), the largest maternity unit in Glasgow, which provides obstetric care to the majority of drug-misusing women in the city.

Eligible infants were born to drug-misusing mothers prescribed substitute methadone in pregnancy and delivered at or admitted within 48 hours of birth to the PRM. Exclusion criteria were prematurity (defined as <36 completed weeks of gestation), congenital ocular abnormality, other significant congenital abnormalities and significant neonatal illness.

For comparative purposes, 50 non-maternal drug-exposed infants were recruited with exclusion criteria identical to the cases. To correct for any potential confounding effect of birth weight, gestation or socio-economic status on the newborn infant VEP, the infants were matched as follows: completed week of gestation, birth weight ± 250 grams, DEPCAT socio-economic group ± 1(123).

The study was approved by Glasgow Royal Infirmary Research Ethics Committee prior to study commencement (REC reference number 08/S0704/40). The study was also granted overall management approval for Greater Glasgow and Clyde Health Board prior to commencement (Research and Development Project number YN08NN325). All aspects of the study were conducted in accordance with Good Clinical Practice guidelines.

Neonatal recruitment commenced on 15th October 2008 and ended on 30th March 2010. Six month follow-up commenced on 21st April 2009 and ended on 28th September 2010.

Design: Prospective cohort study.

3.4 Sample size calculation

Pilot study 1 demonstrated a significant difference in flash VEP latency, amplitude and morphology between 21 methadone-exposed infants and 20 control infants (122). These infants were however exposed to poly-drug misuse. The aim was to recruit enough patients to determine the independent effects of different drugs of misuse as well as substitute methadone on the neonatal VEP. From local audit and research data it was estimated that approximately 20% of pregnant women prescribed substitute methadone would use no additional illicit substances (126). It was therefore calculated that 100 maternal drug-exposed infants would require to be recruited to identify a sub-group of 20 who had been exposed to methadone alone, thereby permitting study of the isolated effect of methadone on the neonatal VEP. In addition, to minimise the confidence intervals of parameters such as VEP amplitude and latency the largest feasible number of infants was recruited within the two year duration of the study. From local audit, it was predicted that approximately 150 babies per year would be delivered at PRM to mothers prescribed substitute methadone, of whom around 120 would be delivered at $\geq$36 weeks gestation. Based on previous studies, a 65% recruitment rate predicted around 70 eligible infants recruited each year. This was a conservative estimate as recruitment rate in the pilot study was 84%. To allow sufficient time for completion of the six month follow-up an eighteen month recruitment period was planned, anticipating recruitment of 100 maternal drug-exposed infants to the study. Drop-out was predicted to be minimal for Part 1 of the study since all intervention was neonatal.

From this cohort, a sub-group of around 50 (50%) infants was expected to develop significant NAS requiring pharmacological treatment (4,126). A group of this size was likely to demonstrate 95% confidence intervals for averages of neonatal flash VEP parameters of around ±2.3μV and ±10.7ms for P2 amplitude and latency (78), which is adequately narrow for clinical purposes. To match this study group in size, it was proposed to recruit the same number of non-maternal drug-exposed infants (n=50).

Follow-up: Local audit demonstrated that approximately half of all methadone-exposed infants offered follow-up clinic appointments failed to attend on two or

more occasions (126). Follow-up studies of developmental outcome in infants exposed to opiates *in utero* report drop-out rates varying from 10-60% (38,45,47). A 40% drop-out by six months was therefore assumed, giving 60 drug-exposed infants and 30 comparison infants for visual and neurodevelopmental assessment at this age. A well-accepted test battery of child development for examining functional vision (127) used a sample size of 28 to define normal results at around six months: these data gave confidence to describe adequately both groups with the predicted study numbers.

3.5 Recruitment

Eligible mothers and babies were identified by daily communication with the midwifery and medical staff on the postnatal wards of the PRM. All mothers were approached in person after discussion with the attending midwife. Mothers were given verbal and written details of the study and time to consider participation and to discuss the study with their partner (Appendix 1 - Parent Information Sheet). If verbal consent was given, the mother was asked to sign a consent form and was given a copy of both the consent form and Parent Information Sheet to keep. A copy of the consent form was inserted in the baby's notes and a record kept for the study document folder. Consent for the six month follow-up was also obtained at this time, and maternal contact details recorded. A letter was subsequently sent to the General Practitioner to inform them of the infant's participation in the study (Appendix 2 - Letter to GP).

3.6 Data collection

3.6.1 Maternal data

Information regarding the pattern of drug misuse in pregnancy was obtained from mothers of both drug-exposed and comparison infants by confidential interview and by review of maternal case notes. Mothers were also asked about their smoking habit and alcohol use in pregnancy. The interview was conducted with the mother alone, and it was stressed that information disclosed would not affect the care that either the mother or her baby would receive.

Further maternal data collected included age, body mass index (BMI), prescription of antidepressants or antipsychotics during pregnancy and socioeconomic group. Socioeconomic group was defined using the Carstairs DEPCAT scoring system (123). This scoring system uses information from the 2001 Census to place geographical areas into socioeconomic groups based on the following: presence of overcrowding, unemployment, social class (based on occupation) and car ownership. The report provides a DEPCAT score for each postcode in Scotland with a score of one representing the most affluent areas and a score of seven representing the most socially deprived areas.

3.6.2 Neonatal data

Data collected included birth weight, sex, gestation, method of delivery, Apgar scores at birth, occipito-frontal circumference (OFC) and method of feeding. Most of these data were collected from maternal and neonatal case notes. The OFC was measured with a disposable measuring tape three times in succession by the researcher and the largest value plotted on an appropriate growth chart (Four-in-one decimal growth charts, Designed and published by Child Growth Foundation, Harlow Printing Limited). The closest centile line for both birth weight and OFC was documented. Low birth weight (LBW) was defined as birth weight < 2500g, and small for gestational age (SGA) was defined for infants whose birth weight was < 3rd centile for gestational age.

The presence and severity of NAS was recorded throughout the infant's hospital stay, including requirement for and duration of pharmacological treatment. NAS severity was assessed as per routine practice at PRM, using a modified version of the Lipstiz scoring tool (Appendix 3 - PRM neonatal abstinence syndrome guidelines) (28). Infants underwent twice daily NAS scoring by trained midwifery staff on the postnatal ward. Treatment was commenced if infants had two NAS scores ≥5 and they were unable to be settled between feeds. Treatment was commenced as per protocol with oral morphine solution at a dose of 60 micrograms/kg/dose four hourly and increased if necessary in increments of 10 micrograms/kg/dose to a total of 80 micrograms/kg/dose four hourly. Infants whose symptoms remained significant on the maximum dose of oral morphine solution, or whose symptoms worsened after weaning of oral morphine solution,

were commenced on oral phenobarbital solution. Length of hospital stay and need for admission to the Neonatal Unit were recorded.

3.6.3 Confidentiality and data protection

All patient related information was stored on a password protected study database. At study entry, each participant was allocated a unique study identification number and all personal data were stored anonymously on the study database under this number. A list matching study number to patient identification was stored separately on a password protected computer.

3.7 Toxicology

3.7.1 Toxicology samples

Most methadone-prescribed mothers had routine urinalysis performed at their booking hospital visit and at approximately 36 weeks gestation. Additional urine toxicology samples were obtained in some mothers during their pregnancy, on clinical grounds. A specimen of the infant's urine and a sample of meconium were obtained as soon as possible after delivery (but after informed consent had been obtained), to facilitate accurate assessment of the pattern of drug exposure *in utero*. Urine was obtained via a urine bag applied to the infant's perineum, and was sent to the laboratory in a universal container. Meconium was obtained directly from the infant's nappy and collected into a universal container. Samples of each were frozen at -20°C prior to being analysed in batches. Meconium samples were also obtained from comparison infants after study recruitment and screened for alcohol metabolites.

3.7.2 Toxicology analysis

Maternal and infant urine samples were analysed by the regional toxicology laboratory at Gartnavel General hospital using Abbott enzyme multiplied immunoassay technique (EMIT) assays run to Substance Abuse and Mental Health Services Administration guidelines on an Abbott Architect Analyser. Assays

included methadone, opiates, benzodiazepines, amphetamines, cannabinoids and cocaine metabolites.

Meconium samples were analysed in the Department of Forensic Medicine and Science at the University of Glasgow. Established methods for drug testing in meconium involved enzyme-linked immunosorbent assay screening plus solid phase and liquid-liquid extraction followed by gas chromatography-mass spectrometry. These procedures allowed detection of the major drug groups, including methadone and detected elevated fatty acid ethyl esters as a biomarker for prenatal alcohol exposure (17,128). A cut-off level of ≥ 10,000 nanograms/gram was used to signify excessive alcohol consumption in pregnancy (19).

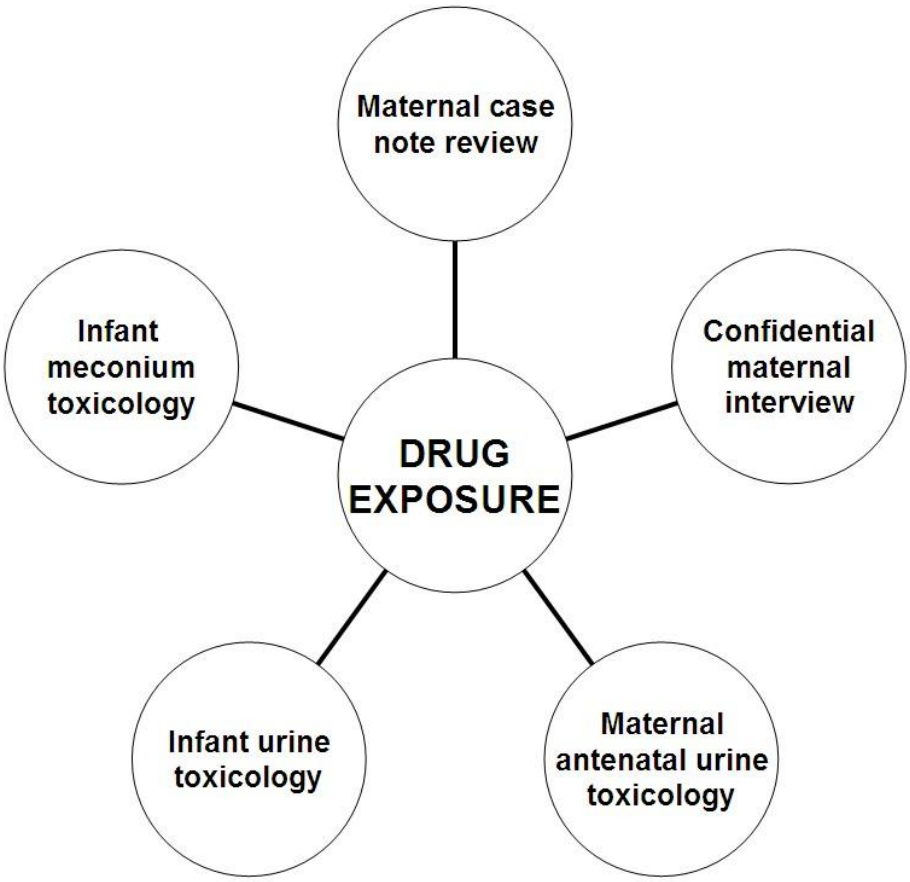


Figure 3-1 Infant drug exposure
A comprehensive assessment of drug exposure was undertaken in infants exposed to methadone *in utero* using the methods summarised above.

3.8 Neonatal VEP recording

VEPs were recorded within 72 hrs of birth in accordance with a Standard Operating Procedure (Appendix 4 – Standard Operating Procedure for recording VEPs). They were recorded from the occipital scalp using three silver-silver-chloride electrodes placed according to international 10:20 classification. VEPs were recorded to single flashes of light and then to flicker stimuli. All stimulation was binocular. Light stimuli were delivered using a hand held LED stimulator. Stimulus generation, recording and data storage were carried out using the Espion® evoked potential system (Photograph 1). Impedance was recorded at the start and end of the recording and aimed to be < 10 kOhms.

3.8.1 Flash VEPs

A white pulse flash light stimulus was delivered at a frequency of 1 Hz, at two different luminance: three candelas seconds per meter squared (cds/m^2) (standard flash) and 28 cds/m^2 (bright flash) (Photograph 2). A minimum of 30 and up to 100 trials were undertaken and repeated to ensure reproducibility. All VEPs which were described as non-detectable had 100 trials repeated twice. The sample frequency was 1000 Hz. The standard flash (subsequently referred to as dim flash) was chosen as it represented International Society for Clinical Electrophysiology of Vision (ISCEV) standards for flash VEPs in adults (75). The bright flash was selected based on data from Pilot study 1 which demonstrated significant differences between groups when a bright flash stimulus was used (Chapter 2.1).

3.8.2 Flicker VEPs

Pulse and sine wave flicker stimuli were presented at 4.64 Hz, 5.86 Hz, 7.32 Hz, 12.7 Hz and 18.55 Hz. These frequencies were chosen based on pilot normative control data which demonstrated an optimal flicker response at a frequency of 4.64 Hz in newborn infants (Chapter 2.2). A bright luminance flicker stimuli was used (500 cd/m^2), also based on the pilot study. Each neonatal VEP recording took approximately 45 minutes. Sleep state and eye-opening during recordings

were noted. Sleep state was defined as awake, drowsy or asleep and eye opening state as open, intermittent or closed.

3.9 Follow up assessment

All infants were invited to participate in assessment at six months of age. Contact details were recorded at recruitment. When the infant was approximately five months old an initial invitation to attend for follow up was issued: if the parent was not contactable at this stage via the recorded details, contact tracing was undertaken by contacting the general practitioner and/or patient services. If the infant was no longer in the care of the biological parents, social work was contacted to trace the infant's whereabouts. An appointment was made over the telephone for follow-up attendance, with every effort made to ensure that the timing suited the parents/carers' schedules and the child's routine. Where applicable, follow-up was co-ordinated with existing scheduled hospital out patient clinic appointments. A letter was sent as confirmation of the agreed appointment date (Appendix 5 – Letter to parent/carer) and a reminder telephone call was undertaken on the day prior to the appointment.

To facilitate attendance, a taxi was made available to transport the parent/carer and child to the hospital. Follow-up was performed at the PRM and included: 1) repeat VEP testing, 2) clinical visual assessment, 3) developmental assessment and 4) growth parameter measurement.

3.9.1 VEP testing

VEP testing at six months of age was undertaken using pattern-onset stimuli (Photograph 3). Pattern onset stimuli produce larger and clearer VEPs in patients with nystagmus compared to pattern reversal stimuli (106) and pilot data (Chapter 2.4) had demonstrated a high incidence of nystagmus in a drug-exposed study group. VEPs were recorded using the standard clinical montage of three electrodes as described in the SOP. Pattern-onset VEPs were recorded to the appearance of 60' (one degree of arc) black and white checks alternating between a diffuse grey background with equal overall luminance presented at one reversal per second. The 60' check size was chosen to comply with ISCEV

standards for clinical recordings of transient pattern VEPs (75). Depending on response to the 60’ check size, further responses were investigated at 15’ (0.25 degree of arc) and 120’ (2 degree of arc) checks (Figure 3-2). Six month old infants with normal vision were expected to demonstrate a VEP response at the small (15’) check size (92). The infant’s attention was maintained on the computer screen by tapping the fixation screen and dangling rattles and bells; the recording was paused if the infant lost fixation. As with flash VEPs, a minimum of 30 and up to 100 trials were recorded and repeated to ensure reproducibility for each check size.

As the follow up study progressed it became apparent that only a minority of infants were presenting with nystagmus, and a publication subsequent to determination of the study protocol demonstrated that the pattern-reversal VEP was delayed in four month old infants who had been exposed to methadone *in utero* (121). It was therefore decided to add pattern-reversal VEPs to the visual electrophysiology testing protocol for infants who had not yet undergone follow up. Pattern-reversal VEPs were recorded to a black and white checkerboard at 100% contrast with 60’ check sizes (one minute of arc) and presented at one reversal per second.

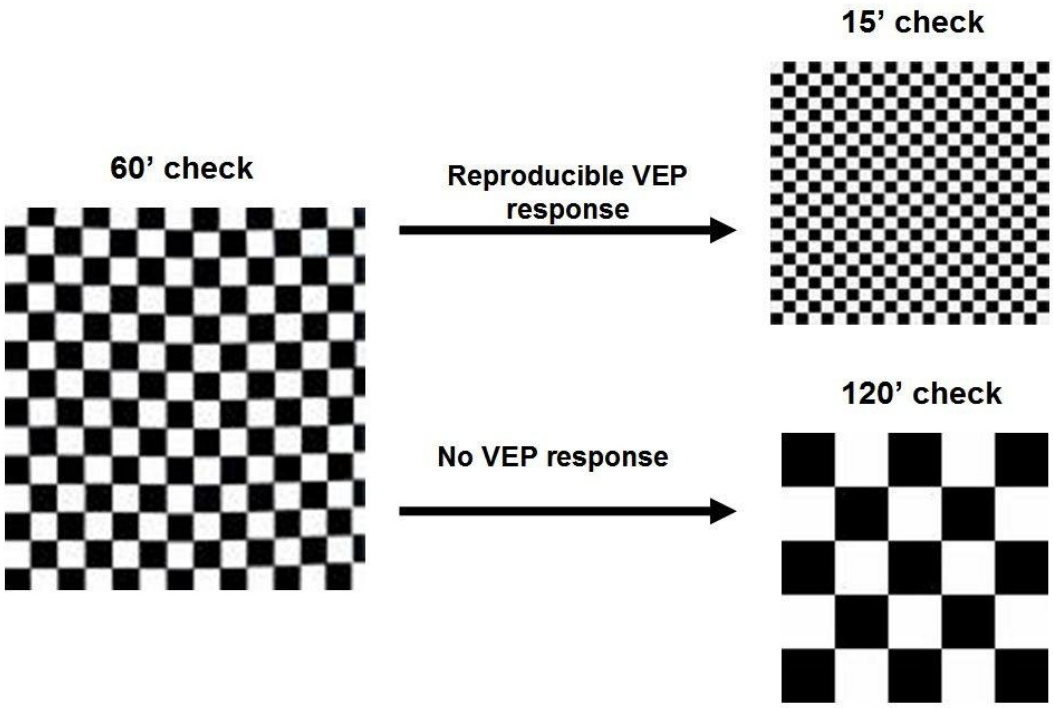


Figure 3-2 Pattern VEP testing
All 6 month old infants were tested with a 60’ check size. Infants with a VEP response were then tested with a smaller (15’) check size. Infants with no response at 60’ were tested with a larger (120’) check size. Infants who remained compliant and attentive had all three check size VEPs recorded.

3.9.2 Clinical visual assessment

Clinical visual assessment used a modification of the Atkinson test battery of child development for examining functional vision (Appendix 6 – Standard Operating Procedure for visual assessment) (127). This test battery provides normal data for basic visual capacities as well as specific visual functions in perceptual, visuo-motor and spatio-cognitive domains. Vision tests used included: pupil responses, diffuse light reaction, lateral tracking, corneal reflexes, lateral field testing by peripheral refixation, convergence of eyes to an approaching object, defensive blink to an approaching object, visual following of a falling toy, batting and reaching, screening retinoscopy and Cardiff acuity cards for preferential looking. The test battery provided criteria for a pass or fail in each test and recommendations for ophthalmology referral.

Pilot work demonstrated that the most commonly identified visual abnormalities in infants exposed to methadone and other drugs of misuse *in utero* were reduced visual acuity, nystagmus, delayed visual maturation, strabismus and refractive errors (Chapter 2.4) (125). These were all covered by the visual screening test as follows:

- Cardiff acuity test cards to assess acuity and screen for DVM (Photograph 4),

- Observation (including covering each eye in turn) and lateral tracking to observe for nystagmus,

- Corneal reflexes to observe for strabismus,

- Screening retinoscopy to screen for significant refractive errors.

3.9.3 Neurodevelopmental assessment

Neurodevelopmental assessment was undertaken using the Griffiths Mental Development Scales for babies from 0 to 2 years (1996 revision) (Appendix 7 - Standard Operating Procedure for neurodevelopmental assessment). This provided a general developmental quotient (GQ) plus five subscales (locomotor; personal/social; hearing and language; eye and hand co-ordination; performance) (Photographs 5 and 6). A fail was defined as a GQ <85.

3.9.4 Growth parameters

Infants who attended follow-up were weighed using professional paediatric scales (BD-815 MA paediatric weighing scales, Tanita Corporation) and their OFC was measured. Growth parameters were plotted on sex-appropriate growth charts. Further data collected included infant feeding and details of any illnesses and/or hospital visits.

All assessments had to be co-ordinated with the child's nap, feeding times and limited periods of co-operation. Full assessment took approximately one to one and a half hours. Priority was given to the clinical visual assessment and pattern onset VEP testing in infants with limited co-operation.

Any babies who failed to reach required standards for visual or neurodevelopmental progress were referred to an appropriate specialist (consultant paediatric ophthalmologist or consultant neonatologist) for further assessment.

All drug-exposed infants seen at the six month follow-up were offered a formal ophthalmology out-patient clinic appointment irrespective of whether visual concerns were identified (Appendix 8 - Letter to ophthalmology). This was done at the request of the consultant ophthalmologist involved in the study who judged that sufficient concern existed regarding long-term visual outcome in these infants to warrant the offer of formal follow-up. It also offered independent verification of findings at the six-month study visit. Since the ophthalmology service was offered on clinical grounds, it was not offered to comparison infants. Assessment at the ophthalmology clinic included cover tests,

ocular motility, visual acuity, cycloplegic refraction and dilated fundoscopy and was undertaken at the Royal Hospital for Sick Children, Glasgow.

3.10 Data analysis

3.10.1 VEPs

3.10.1.1 Flash VEPs

Flash VEPs were categorised as present or absent. When present, the amplitude and latency of peaks and troughs were measured. Peaks and troughs were defined as: P1= any positive component prior to P2, N2= any negative component prior to P2, P2= positive component between 126-300ms and preceding N3 if present, N3= negative component between 200-400ms following the P2 if present, P3= positive component following the N3. Amplitudes were recorded between peaks and troughs or from baseline if there was no preceding peak or trough. The total sum amplitude was calculated as the sum of all recordable peaks and troughs.

Each flash VEP was also placed into a descriptive category of waveform morphology as defined in the pilot study of methadone exposed infants (Chapter 2.1) (122) and the pilot study of VEPs in moderately preterm infants (Chapter 2.3). A new category was included as it was recognised that many of the infants had a more mature response with a P1 component in addition to a P2 component. Descriptive categories were: *typical* (predominant positivity near 200ms (P2), no P1 present), *mature* (P1 and P2 present), *immature* (predominant negativity near 300ms (N3): either no P2 present or N3 amplitude > 3 times P2 amplitude) and *atypical* (response present but unusual waveform which did not meet criteria of other categories). Examples of VEPs from each category are shown in Figures 3-3 to 3-7.

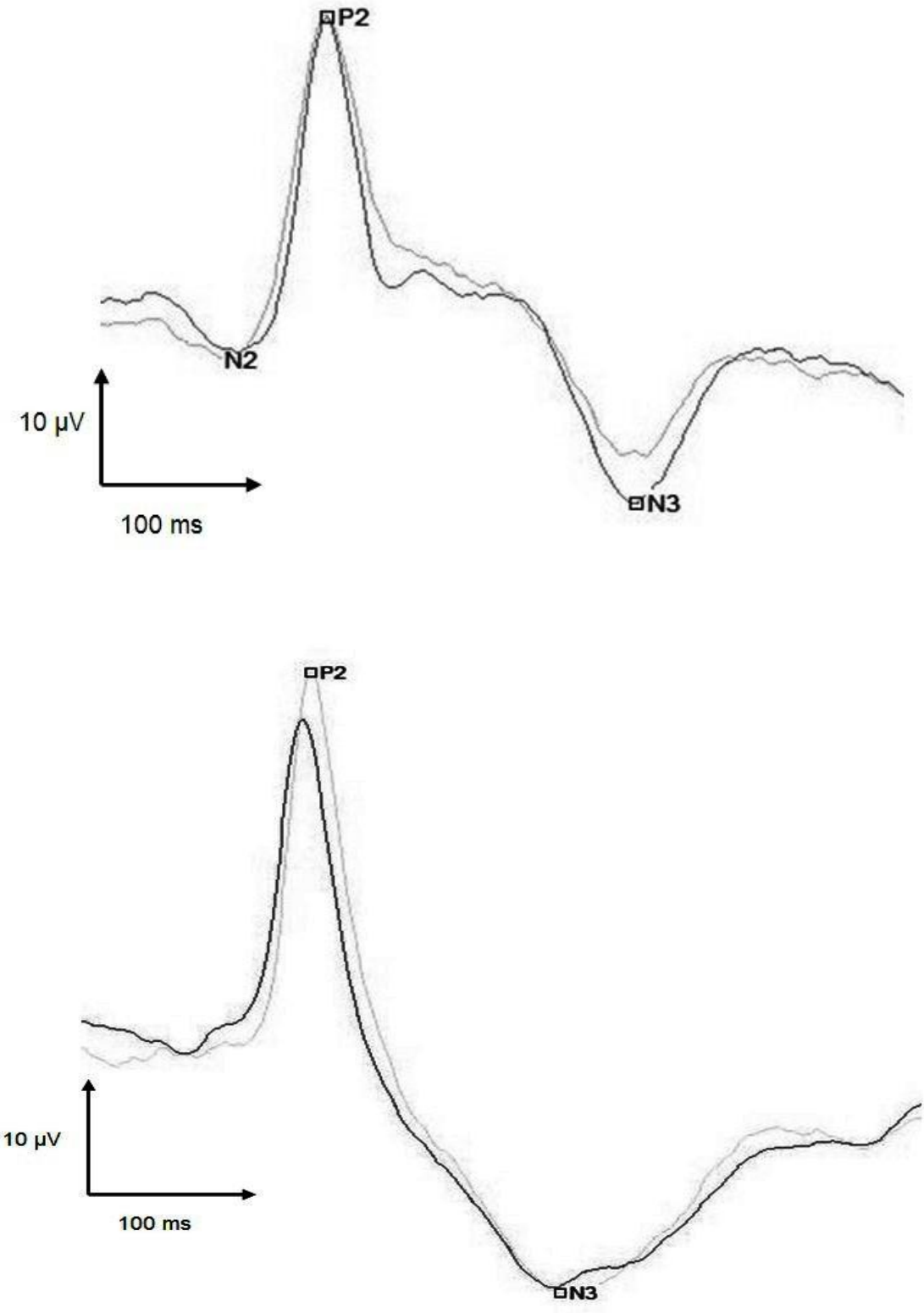


Figure 3-3 *Typical* flash VEP response
Predominant positivity (P2)

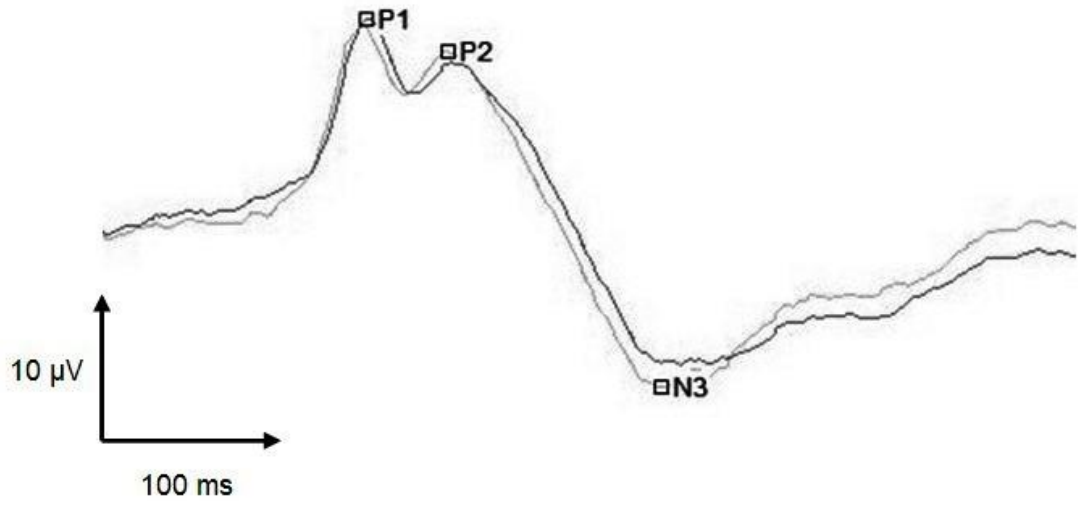


Figure 3-4 *Mature* flash VEP response
P1 and P2 components present

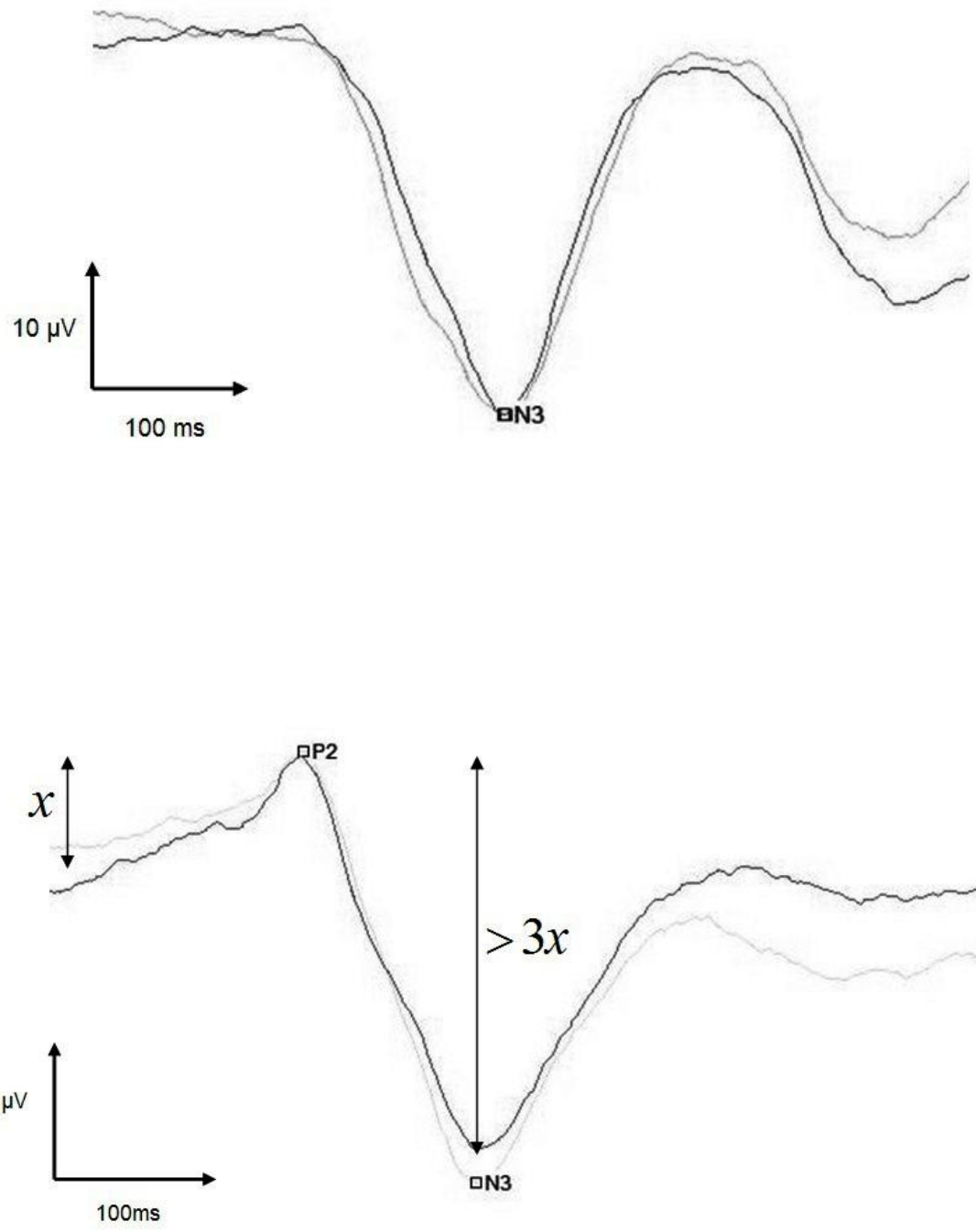


Figure 3-5 *Immature* flash VEP response
The waveforms were predominantly negative. When a P2 response was present, the N3 amplitude was greater than three times the amplitude of P2.

Figure 3-6 *Atypical* flash VEP response
Unusual waveform with a late positive response at 356ms (P3) and no preceding P1 or P2 component.

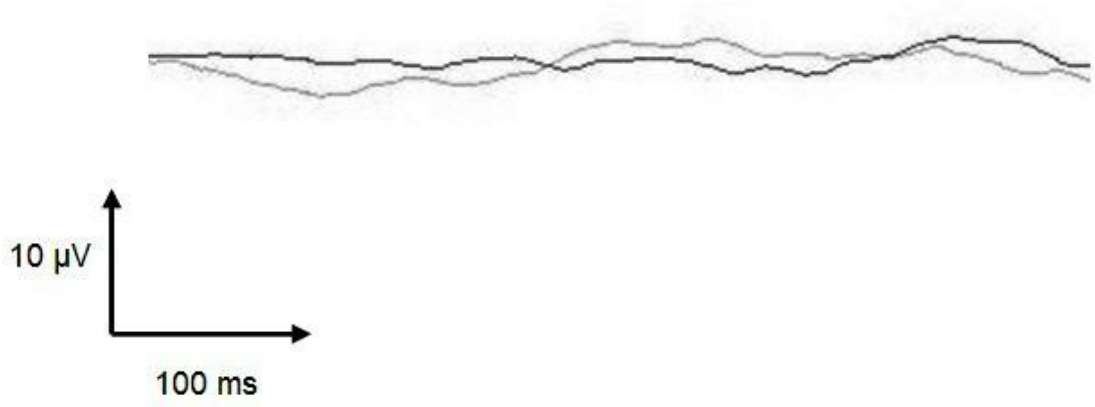


Figure 3-7 *Non-detectable* flash VEP response
No reproducible VEP present

3.10.1.2 Flicker VEPs

Each averaged flicker VEP was subjected to resampling and Fourier analysis. The Meigan and Bach technique was employed to relate the statistical significance of a response to the signal-to-noise ratio (84,85). This technique involved making an estimation of the surrounding noise from the two neighbouring frequencies of the stimulus frequency (one above and one below the response frequency). Using this technique, the statistical significance of the response signal is related to a specific signal-to-noise ratio (SNR): SNR= 2.82, p= 0.05; SNR= 4.55, p= 0.01; SNR= 8.40, p= 0.001 (i.e. the response signal must be 2.82 times the surrounding noise signal to be significant).

A significant response was defined as a signal to noise ratio (SNR) of > 2.82 which corresponds to a p value < 0.05 (85). A significant response at each flicker frequency was sought for the fundamental response (F1) and the F2 and F3 harmonic. When a response was present, its magnitude and SNR were measured. The optimal stimulus frequency was described for each recording, defined as the frequency that elicited the highest amplitude response at F1.

3.10.1.3 Pattern VEPs

Pattern onset VEPs were categorised as present or absent at each of the check sizes tested (15', 60', 120'). When present, the amplitude and latencies of C1 and C2 were recorded. It was recognised that most infants had a C2 peak response but that the morphology of the peak varied in being a) single peak, b) plateau, or c) bifid peak. For consistency, the latencies of the C2 response were defined as follows: a) single peak response: C2 latency at highest point of peak, b) plateau response: C2 latency at start of plateau, and c) bifid response: split into C2a latency, first peak or shoulder and C2b latency, second peak or shoulder (Figure 3-8).

Pattern reversal VEPs were categorised as present or absent at the 60' check size. When present the latencies of N75 and P100 were recorded. Examples of pattern VEPs in each category are shown in Figures 3-8 and 3-9.

All VEPs were analysed by myself and independently by a second assessor (Dr R Hamilton) with extensive experience in paediatric visual electrophysiology. The second assessor was blinded to the infant's group and clinical progress. If there was any discrepancy in analysis of the VEP a third independent assessor (Professor DL McCulloch) reviewed the VEP, blinded to both infant group and clinical progress.

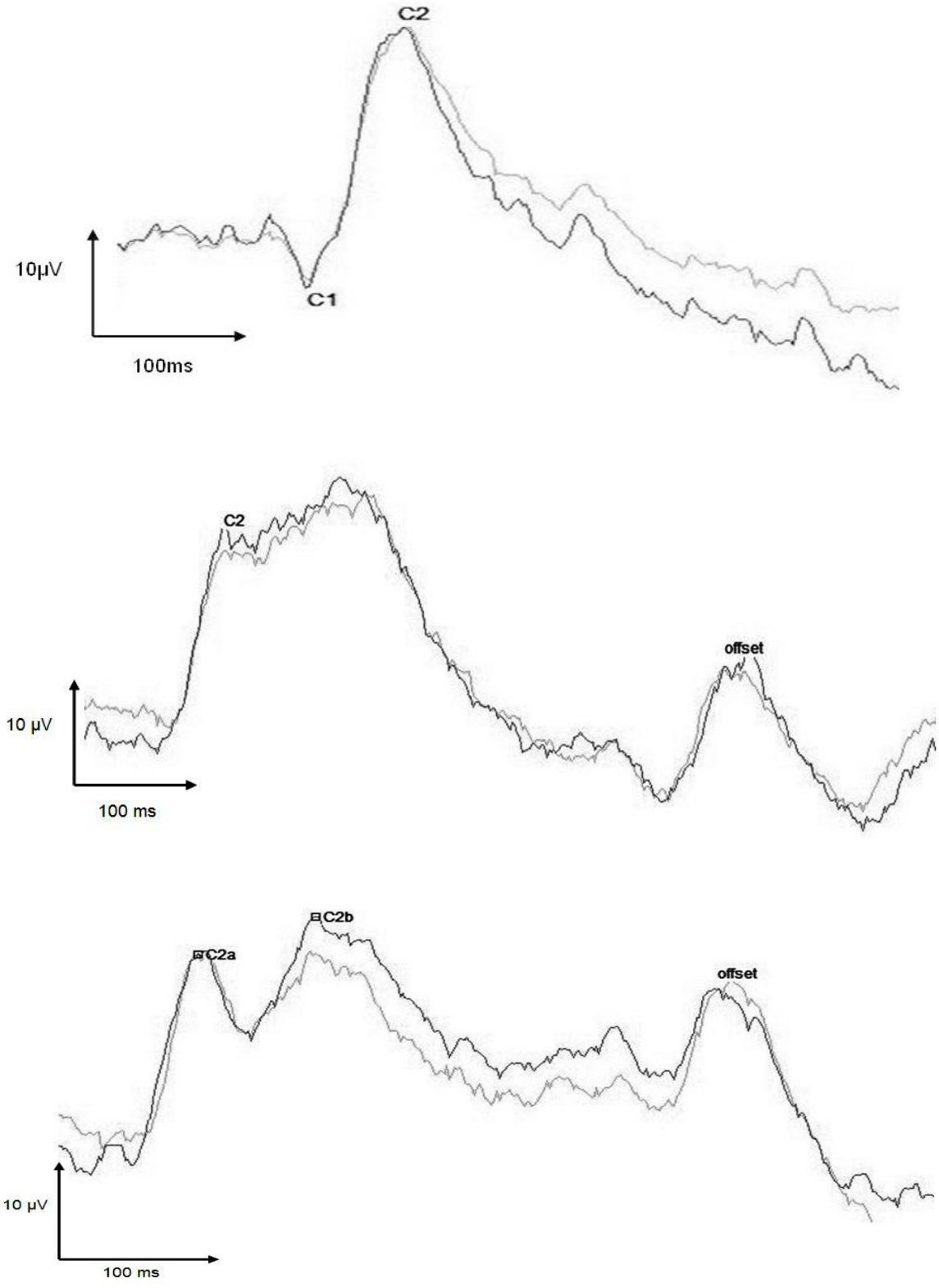


Figure 3-8 Infant pattern onset responses
a) single peak, b) plateau, c) bifid peak

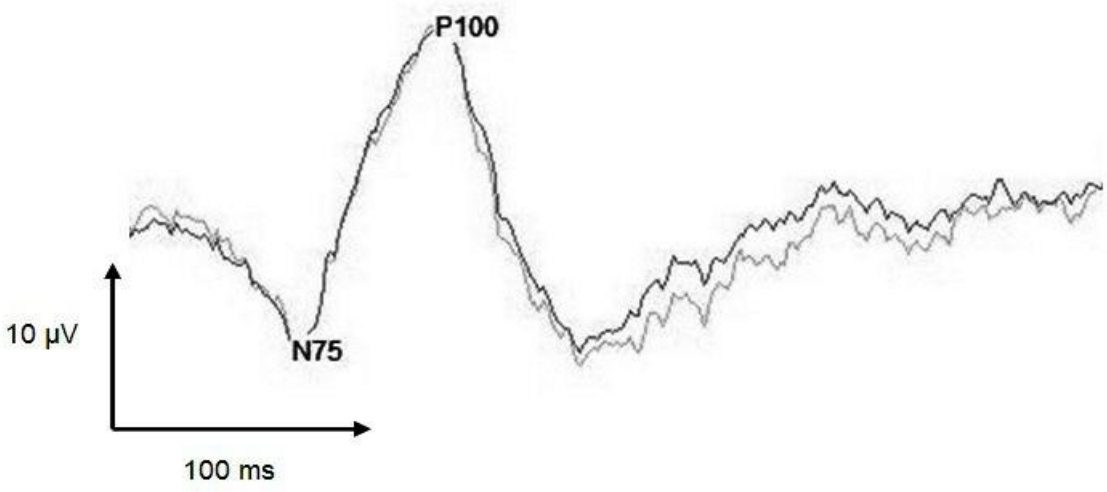


Figure 3-9 Infant pattern reversal response

3.10.2 Drug exposure

The dose of methadone prescribed to the mother at delivery was noted. For other drugs of misuse, positive exposure was defined if either toxicology samples or maternal history were positive. It was assumed for the purpose of the study that toxicology samples could provide a false negative but not a false positive result and therefore if any sample tested positive this confirmed exposure to the drug under study. For example, if a mother denied cocaine use and mother and infant urines were negative for cocaine but meconium was positive, this counted as a positive exposure to cocaine. If a mother admitted amphetamine use but all toxicology samples were negative for amphetamine, this counted as a positive exposure to amphetamine. Drug exposure was then divided into the following categories: group 1) opiate exposure alone, group 2) opiate and cannabinoid exposure, group 3) opiate and benzodiazepine exposure (methadone + opiates + benzodiazepines or methadone + benzodiazepines), group 4) opiate, benzodiazepine and cannabis exposure, and group 5) other poly-drug exposure which included stimulants (cocaine and/or amphetamines).

Excessive alcohol consumption during pregnancy was defined as a total FAEE concentration in neonatal meconium greater than 10,000 nanograms/gram. The group of infants who had been exposed to excessive alcohol during pregnancy was compared to those infants not exposed, and excess alcohol exposure was included in multivariate regression analysis.

3.10.3 NAS

NAS was categorised into four groups: 1) no NAS: infants with NAS scores ≤ 3 in the first week of life and who did not require pharmacological treatment, 2) mild NAS: infants with NAS scores > 3 in the first week of life and who did not require pharmacological treatment, 3) moderate NAS: infants who required standard treatment for NAS as per unit policy (maximum dose of 60 microgram/kg/dose oral morphine solution only) and 4) severe NAS: infants requiring second line treatment for NAS (either increased dose of oral morphine solution or addition of phenobarbital).

3.11 Statistical analysis

Numerical data were described as means and standard deviations (SD) for normally distributed data and medians and interquartile ranges (IQR: Q1, Q3) for non-normally distributed data. Distribution of numerical data was determined using Anderson-Darling tests for normality and data were also plotted on histograms and boxplots.

Demographics:

Between group comparisons for normally distributed numerical data (birth weight, OFC, maternal BMI) were analysed using 2 sample t-tests. Between group comparisons for ordinal or ordered nominal categorical data (Apgar scores, DEPCAT score, gestation) were analysed using Mann-Whitney tests. Between group comparisons for nominal and binary categorical data (gender, method of delivery, method of feeding, maternal smoking) were analysed using Chi-squared tests.

VEPs:

VEP latencies and amplitudes were compared between drug-exposed and control groups using 2 sample t-tests / Mann-Whitney tests depending on distribution of data. VEP analysis involving proportions, such as presence of a response or presence of individual components were undertaken using Z test for two proportions or Chi-squared tests.

VEP morphology (mature, typical, immature) was compared between groups using Chi-squared tests for nominal data.

Flicker VEP responses were compared between groups using Z tests for two proportions. Flicker amplitudes were compared using Mann-Whitney tests. As multiple flicker analysis was undertaken, a multivariate, repeated measures, logistic regression model was applied to the flicker response data to compare cases and controls.

Sub-group analysis:

Data were divided into sub-groups depending on drug exposure (group 1-5 outlined above). Kruskal-Wallis tests were used for sub-group analysis of numerical variables and Chi-squared tests were used to compare VEP morphology between groups.

NAS:

VEP latencies and amplitudes were compared between infants who developed NAS requiring pharmaceutical treatment and those who did not using 2 sample t-tests / Mann-Whitney tests depending on distribution of data. VEP morphology was compared between infants who developed NAS and those who did not using Chi-squared tests.

NAS was further sub grouped into four categories as outlined above and Kruskal-Wallis tests were used to investigate differences between sub groups. Morphology between the four sub groups was compared using Chi-squared tests.

Potential confounders:

To assess for potential confounders, demographics were compared between drug-exposed and control infants. Where there was any significant difference identified between groups this predictor variable was entered into a multivariate regression analysis model with each response variable.

Follow-up neurodevelopment:

Griffiths general quotient scores (GQ) were compared between drug-exposed and control infants using Mann-Whitney tests for ordinal data. The sub-quotients for each of the five sub-scales were also compared between groups. A GQ score of <85 was classified as abnormal and the proportion of infants with abnormal neurodevelopment was compared between groups using Z test for two proportions. Linear regression models were used to correct for potential confounders on developmental outcome.

Follow-up visual assessment:

The proportion of infants who failed visual assessment in each group was compared using Chi-squared tests (or Fisher's exact tests where group numbers were small). Neonatal VEP amplitudes and latencies were compared between drug-exposed infants with normal and abnormal visual screening using 2 sample t-tests or Mann-Whitney tests depending on distribution of data. Neonatal VEP morphology was compared between drug-exposed infants with normal and abnormal visual screening using Chi-squared tests.

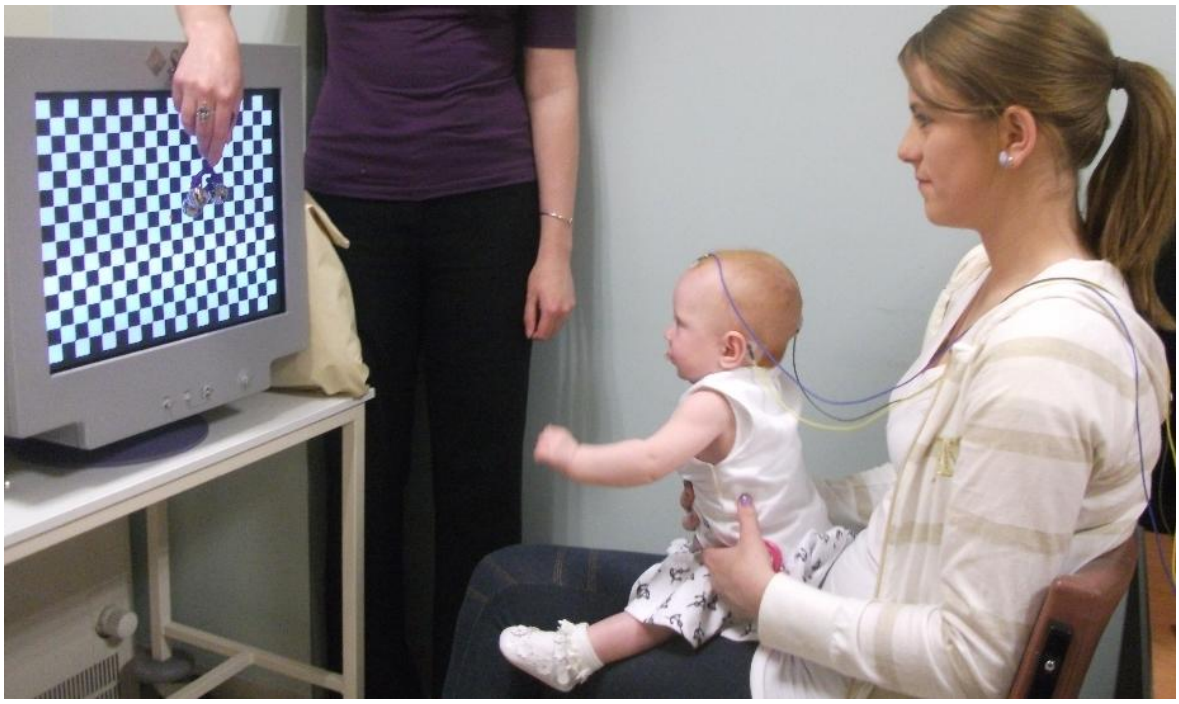
In addition, relative risk and attributable-risk percent were calculated for the outcomes of abnormal VEP and abnormal clinical visual outcome.

All analyses were done using Minitab (versions 15 and 16) with a significance level of 5%. Where available, 95% confidence intervals were quoted.

Photograph 1: Espion evoked potential system (1= Espion recording system, 2= hand held light stimulus for neonatal testing, 3= computer monitor for pattern VEP testing)

Photograph 2: Neonatal flash VEP recording using hand held light stimulus

Photograph 3: Pattern VEP recording in six month old infant (120’ check size)



Photograph 4: Cardiff card visual acuity testing

Photograph 5: Griffiths developmental assessment: eye and hand co-ordination

Photograph 6: Griffiths developmental assessment: locomotor skills

4 Chapter 4 Results: Neonatal data

One hundred and fifty four mothers were approached regarding the study, of whom 152 consented to participation (102 drug-exposed infants; 50 comparison infants), giving a 98% recruitment rate. In all the following graphs and tables, drug-exposed infants are referred to as cases and non-drug-exposed comparison infants are referred to as controls.

4.1 Demographics

Infant and maternal demographic data are shown in Table 4-1. There was no difference in sex or mode of delivery between the groups. The median gestation of both groups was 39 weeks and median 5-minute Apgar score of both groups was nine.

The vast majority of infants in both groups were formula fed (87% of cases and 90% of controls); mode of feeding did not differ between groups.

Although the drug-exposed infants had a marginally lower mean birth weight than comparison infants, this difference was not significant (2892 grams *vs* 3005 grams, 2-sample t test, p=0.209). There was no difference in the proportion of infants in each group who were either LBW or SGA.

Despite similar birth weights, the drug-exposed infants had smaller head circumferences compared to comparison infants. The proportion of infants with microcephaly (OFC < 3rd centile) did not however differ between groups.

A higher proportion of mothers of drug-exposed infants smoked compared to mothers of comparison infants (95% *vs* 60%, p< 0.001). The mean OFC of infants born to smoking mothers was significantly smaller than that of infants born to non-smoking mothers (33.49cm *vs* 34.66cm, p=0.001). After correcting for smoking status using a linear regression model, there was no longer a significant difference in the OFC between groups (p=0.280).

Socioeconomic class tended to be marginally lower in the drug-exposed infants (median DEPCAT 7) compared to the comparisons (median DEPCAT 6), but this was not statistically significant. Maternal BMI did not differ between groups.

Fourteen of the methadone prescribed mothers were on antidepressant or antipsychotic medication during pregnancy (ten on antidepressants alone, two on antipsychotics alone, two on combined treatment), compared to none of the comparison mothers (14/102 *vs* 0/50; Fisher's exact test p=0.005).

Table 4-1 Infant and maternal demographics

	Cases (n=102)	*Controls (n=50)*	*p-value*
Sex (Male)	46%	44%	0.809
Mode of delivery			
SVD	72%	70%	
LUSCS	21%	20%	0.797
Instrumental	7%	10%	
Gestation (wks)	39.3 (38.2-40.1)	39.7 (38.1-41.6)	0.419
5-min APGAR	9 (9-10)	9 (9-10)	0.862
Birth weight (gm)	2892 (505)	3005 (539)	0.209
SGA	18%	20%	0.727
LBW	20%	18%	0.812
OFC (cm)	33.5 (1.56)	34.1 (1.6)	**0.015**
Microcephaly	8%	8%	0.973
Feeding at D/C			
Formula	87%	90%	
Breast	7%	8%	0.507
Mixed	6%	2%	
Maternal smoking	95%	60%	**<0.001**
Maternal BMI	23 (21-26)	23.5 (21-30)	0.293
Maternal DEPCAT	7 (5-7)	6 (4-7)	0.058
Maternal antidepressants /antipsychotics	14%	0%	**0.005**

Data are given as percentage responses. Gestation, Apgar scores, DEPCAT scores and BMI are medians (inter-quartile range). Birth weight and OFC are means (standard deviation). Percentage responses were compared using Chi-squared tests, birth weight and OFC using 2 sample t-tests and gestation, Apgar, DEPCAT and BMI using Mann-Whitney tests. SVD: spontaneous vertex delivery, LUSCS: lower uterine segment caesarean section, SGA: small for gestational age, LBW: low birth weight, OFC: occipito-frontal circumference, D/C: discharge, BMI: body mass index, DEPCAT: Carstairs deprivation index score.

4.2 Neonatal Abstinence Syndrome

Just under half of all drug-exposed infants developed NAS sufficiently severe as to require pharmacological treatment (49/102, 48%). The proportion of infants in each NAS severity group (no NAS, mild NAS, moderate NAS and severe NAS) is shown in Table 4-2.

The median duration of oromorph treatment of the 49 infants treated with oral morphine solution was nine days. 18 of these infants required additional treatment with phenobarbital and the median duration of treatment of this subgroup was 43 days. The median total treatment days were ten days (Table 4-2). All babies treated with phenobarbital were discharged home on the drug with weekly hospital follow-up.

The median hospital stay for the drug-exposed group (n=102) was 9.5 days. Infants requiring pharmacological treatment had a significantly longer hospital stay than infants not requiring treatment (median 13 days *vs* median 6 days, $p<0.001$).

38/102 babies were admitted to NNU (37%). The median length of NNU stay was ten days. The most common reason for NNU admission was ongoing or escalating treatment for NAS (17 infants), however infants were also admitted for respiratory distress (9 infants), poor weight gain or feeding (8 infants) and due to social circumstances (4 infants). 47% of infants were offered a hospital out patient clinic appointment following discharge.

Table 4-2 NAS and admission details for drug-exposed infants

	Cases (n=102)
Treated for NAS	48%
NAS severity	
no NAS	24%
mild NAS	28%
moderate NAS	26%
severe NAS	22%
Oromorph days	9 (8-13)
Phenobarbital days	43 (38-57)
Total treatment days	10 (8-49)
Total hospital stay (days)	9.5 (6-13)
hosp stay with NAS (days)	13 (11-19)
hosp stay no NAS (days)	6 (6-8)
NNU admission	37%
NNU days	10 (5-17)

Data are given as percentage responses or medians (inter-quartile ranges). NAS: neonatal abstinence syndrome, NNU: neonatal unit. NNU days do not include days in post-natal wards.

4.3 Neonatal flash VEPs

Neonatal flash VEP recording was undertaken in 152 infants (102 drug-exposed infants, 50 comparison infants). Two sets of data were lost due to a computer system failure, which left 150 sets of analysable data (100 cases, 50 controls). Age at recording differed by a median of six hours between the drug-exposed and comparison infants (median age of cases = 26 hr, median age of controls = 20.5 hr; p=0.006).

Both sleep state and eye opening state were compared between groups. The sleep state did not differ significantly between groups (cases: 11% asleep, 49% drowsy, 40% awake; controls: 6% asleep, 48% drowsy, 46% awake; Chi ^{2} =1.42, p=0.470). There was also no significant difference in eye opening state between groups with most infants in both groups having their eyes closed during recording (cases: 10% open, 13% intermittent, 77% closed; controls: 14% open, 26% intermittent, 60% closed; Chi ^{2} = 4.97, p=0.090).

4.3.1 Normative control data

Neonatal flash VEPs were recorded in 50 comparison infants. Normative values for these infants are shown in Table 4-3.

Table 4-3 Normative comparison flash VEP data

	Dim	Bright
P1 latency (ms)	117 (108-146)	137 (115-157)
P2 latency (ms)	215 (205-252)	206 (192-229)
N3 latency (ms)	335 (310-353)	321 (250-356)
Total amplitude (μV)	20.4 (11-31)	39.6 (28-67)

Data are medians (inter-quartile ranges).

Associations were sought between VEP latency and amplitude and: gestation, birth weight, OFC, sex and DEPCAT score in the comparison infants.

There was a significant negative correlation between both P1 and P2 latency and gestation (P1 latency: Pearson's correlation coefficient= -0.61, p=0.001; P2 latency: Pearson's correlation coefficient= -0.45, p=0.001) (Figure 4-1). There was no correlation between VEP amplitude and gestational age (Pearson's correlation coefficient= 0.22, p=0.124).

There was no association between VEP latency or amplitude and sex, birth weight, OFC or DEPCAT score.

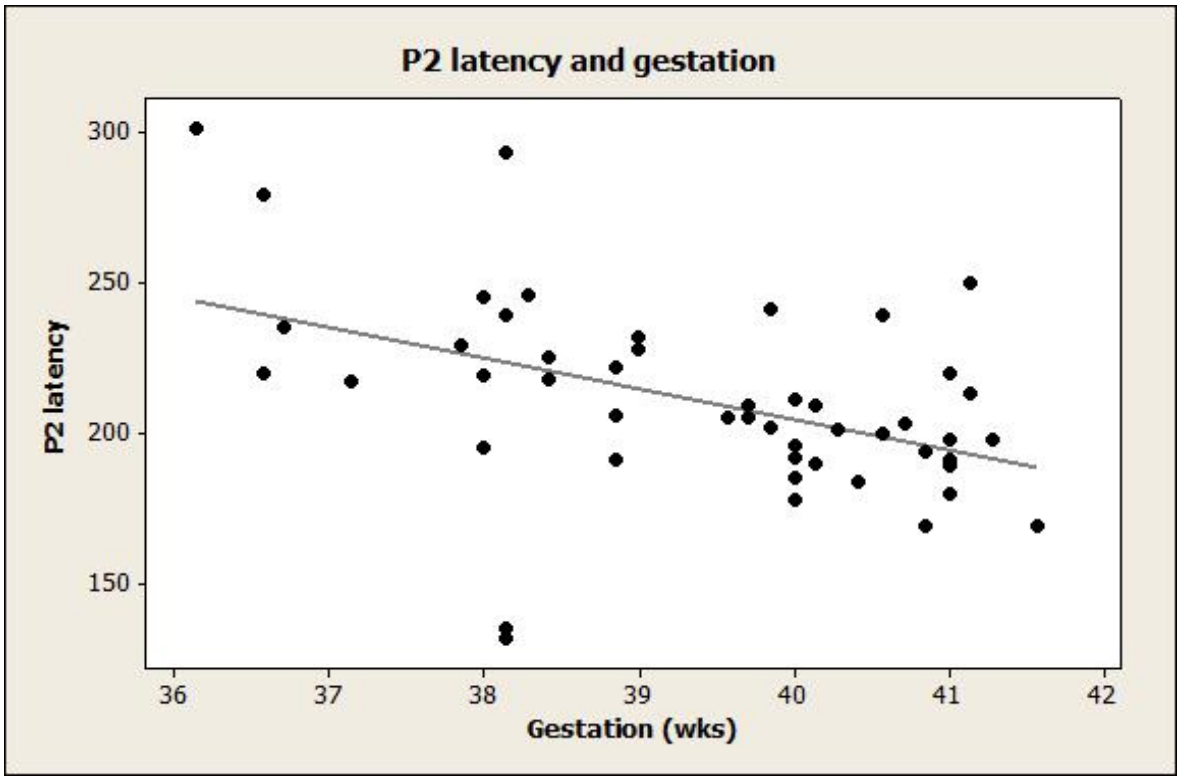


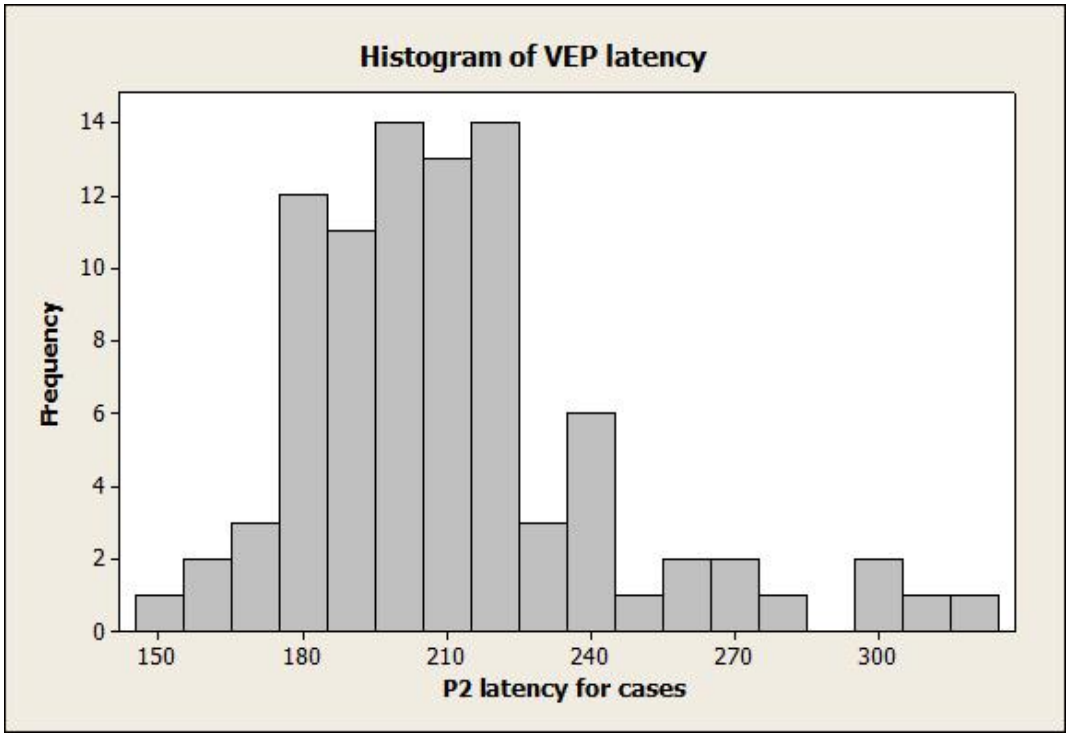
Figure 4-1 Scatterplot of VEP latency and gestational age
There was a significant negative correlation between flash VEP P2 latency and gestational age. R^2 = 20.3%, p=0.001.

4.3.2 Data description and luminance comparison

One hundred and fifty infants underwent both dim and bright flash VEP recording resulting in 300 VEPs for analysis. Prior to statistical analysis the VEP amplitude and latency data were investigated for their distribution using histograms and Anderson-Darling tests for normality. Both types of data appeared to have a skewed distribution (Figure 4-2) and were therefore described as medians and inter-quartile ranges.

The bright flash stimulus consistently produced more VEP components than the dim stimulus. The median latencies were shorter with the bright stimulus compared to the dim and this was statistically significant for the P2, N3 and P3 components (Table 4-4). The bright flash stimulus also produced larger amplitude responses compared to dim: median dim flash amplitude 14.5 (IQR 6.4-28.6) *vs* median bright flash amplitude 31.2 (IQR 19.4-50.3); Mann-Whitney test: 95% CI -20.4 to -12.7; $p<0.001$ (Figure 4-3). The morphology of the VEP waveform differed between the dim and bright flash ($Chi^2 = 50.4$, $p < 0.001$) (Figure 4-4) with the bright flash stimulus producing more mature responses and fewer absent responses.

In summary, a bright flash stimulus produced larger amplitude VEP responses with more components and shorter latencies compared to a dim light stimulus.



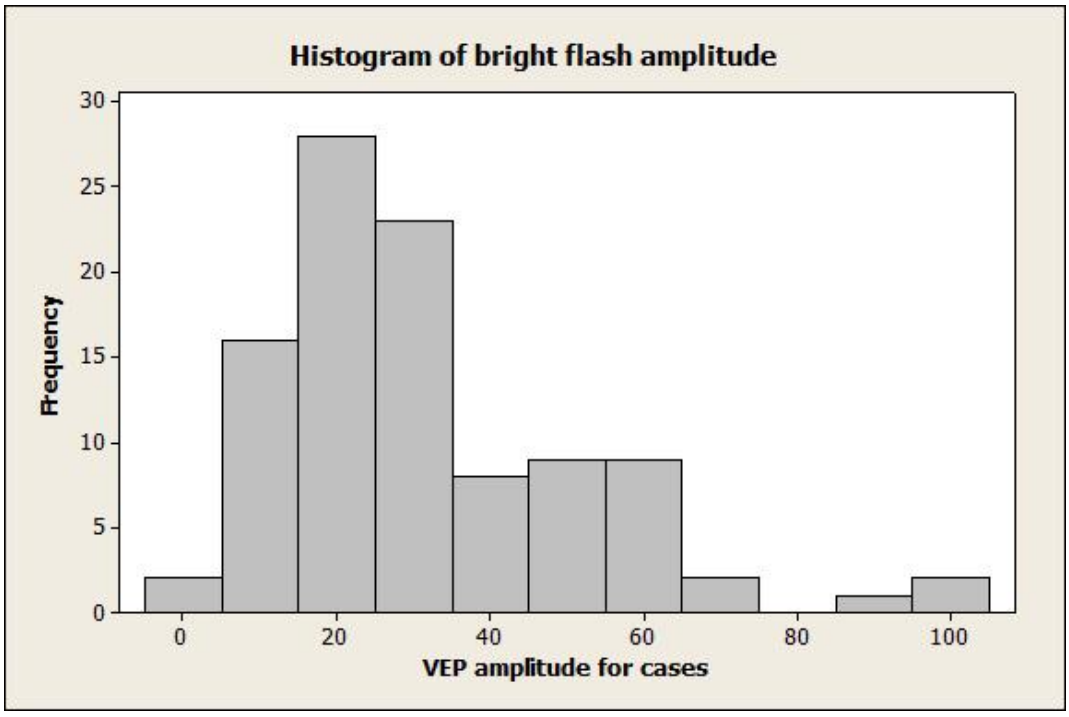


Figure 4-2 Histograms of VEP latency and amplitude
Both VEP latency and amplitude data were of a skewed distribution.

Table 4-4 VEP data description and luminance comparison

	DIM stimulus (n=150)	BRIGHT stimulus (n=150)	p-value
P1 responses	10	45	**<0.001**
P1 latency (ms)	138.5	136	0.458
IQR	117-194	115-202	
N2 responses	20	68	**<0.001**
N2 latency (ms)	170.5	167.5	0.640
IQR	157-200	149-193	
P2 responses	96	138	**<0.001**
P2 latency (ms)	213.5	207	**0.019**
IQR	198-243	192-224	
N3 responses	90	129	**<0.001**
N3 latency (ms)	325	305	**0.004**
IQR	298-354	249-339	
P3 responses	28	60	**<0.001**
P3 latency (ms)	385	323	**0.028**
IQR	312-425	279-387	

Data given are number of responses, medians for latencies and inter-quartile ranges (IQR). The data represents all babies in the study (cases and controls, n=150). Latencies were compared using Mann-Whitney tests and proportion of responses compared using Z test for 2-proportions.

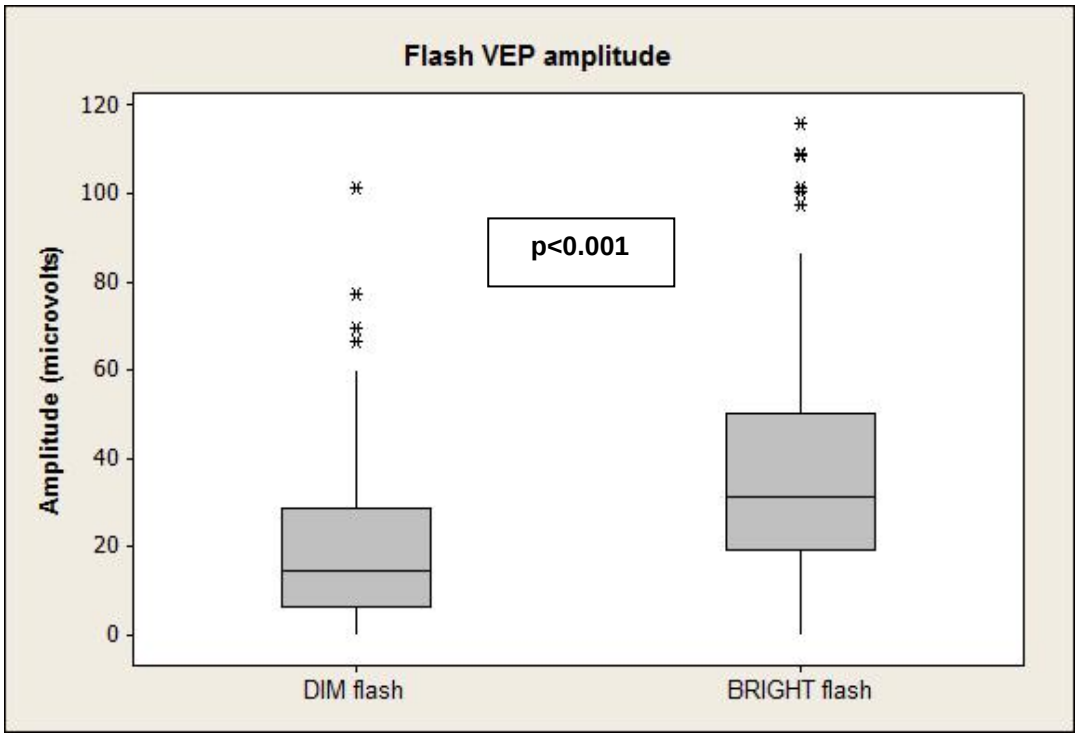


Figure 4-3 Boxplot of VEP amplitude with dim and bright flash
Denotes total sum flash VEP amplitudes using the dim and bright flash stimuli. Horizontal line within the box represents median, upper and lower borders of box represent Q1 and Q3, whiskers represent upper and lower limits and * represent outliers.

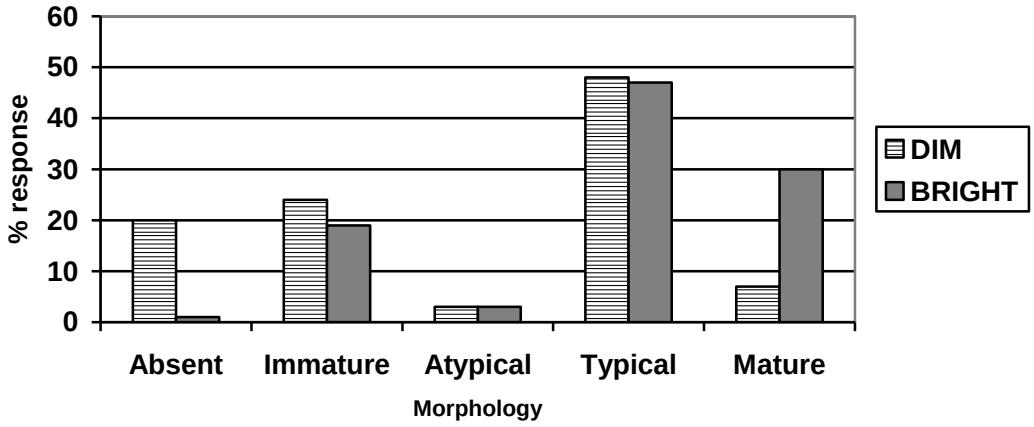


Figure 4-4 VEP morphology with dim and bright flash
The graph demonstrates the VEP morphology with dim and bright flash stimuli. The bright stimuli produced fewer absent responses and more mature responses compared to the dim stimuli: Chi ^{2}=50.4, p<0.001.

4.3.3 Drug-exposed and comparison VEPs

VEP components and latency

The drug-exposed infants had fewer P1 and P2 components present with both the dim and bright flash. Only 53% of cases had a P2 present with the dim flash compared to 86% of the controls ($p<0.001$). In addition, only 21% of the cases demonstrated a P1 response with the bright flash compared to almost half of the controls (48%) ($p=0.001$) (Table 4-5). Significantly fewer drug-exposed infants had a N2 response present than comparisons with the bright flash stimulus: 38% cases *vs* 60% controls, $Chi^2=6.523$; $p=0.011$. Median latencies of the P1, P2, N2 and N3 components did not significantly differ between groups. To further investigate any possible differences in VEP flash latency between groups, the P1 and P2 latency data were logarithm transformed to a normal distribution and subjected to 2-sample t-tests. There was no significant difference in the mean log P1 latency between groups (log P1 latency cases: 4.93 (SD 0.25), log P1 latency controls: 4.91 (SD 0.23), 95% confidence interval -0.119 to 0.168; $p=0.731$). Similarly there was no difference in mean log P2 latencies (log P2 latency cases: 5.34 (SD 0.15), log P2 latency controls 5.33 (SD 0.16), 95% confidence interval -0.052 to 0.054; $p=0.960$).

VEP amplitude

The drug-exposed infants had significantly smaller amplitude responses with both the dim and bright flash compared to the comparisons. The median VEP amplitude with the dim flash for the cases was 11.4 μV (IQR 0-20.5) compared to 20.4 μV (IQR 11.4-31.1) for the controls; Mann-Whitney $p<0.001$ (95% CI –14 to -4.8). With the bright flash stimulus, the median amplitude was 27 μV (IQR 17.1-41.7) for the cases versus 39.5 μV (IQR 28.1-66.6) for the controls; $p<0.001$ (95% CI –20.2 to -6.4) (Figure 4-5).

Flash morphology

Dim flash: VEPs were classified as absent, atypical, immature, typical or mature as defined in the methods section. 27% of drug-exposed infants had an absent VEP compared to 6% of comparisons. For the purpose of statistical analysis,

infants in the atypical group were combined with infants in the immature group. There was a significant difference in the VEP morphology between groups with the drug-exposed infants having more absent and immature/atypical responses, and fewer typical and mature responses (Chi ^{2} 19.1, $p<0.001$) (Figure 4-6).

Bright flash: VEPs were classified in a similar manner to the dim flash, however only 1% of cases had an absent VEP with the bright flash and no comparisons had an absent response. Therefore for the purpose of statistical analysis the absent, atypical and immature groups were combined to form one immature/abnormal group. Again there was a significant difference in VEP morphology between groups with the drug-exposed infants having more immature/abnormal responses and fewer mature responses (Chi ^{2} 13.6, $p=0.001$) (Figure 4-7).

Abnormal neonatal flash VEP characteristics

The comparison infant data were used to define normal flash VEP characteristics. For VEP latency and amplitude, the upper and lower limits respectively of the comparison VEPs were used to define limits of normality. Using these cut off values an abnormal flash VEP was defined as: P2 latency > 276 ms (dim flash) or > 301 ms (bright flash), total amplitude < 5 μV (both flashes), an absent VEP response to either flash or an immature VEP response to the bright flash. Each flash VEP was classified as normal or abnormal using these categories and these data were used to assess the predictive value of the flash VEP in the follow up assessments (Chapter 5 Results: Follow up data).

Relative risk of VEP abnormalities

VEP abnormalities in methadone-exposed and non-exposed infants can be summarised as follows:

	Abnormal VEP	Normal VEP
Methadone-exposed	56	44
Non-exposed	5	45

Relative risk= 56/(56+44) / 5/(5+45) = 5.6.

Therefore, methadone-exposed infants were over five times more likely to have an abnormal VEP than non-exposed infants.

The attributable risk percent was also calculated to estimate the proportion of VEP abnormalities amongst the exposed group which was attributable to methadone exposure.

% AR = incidence in exposed group-incidence in non exposed group/ incidence in exposed group x 100 = 82%.

Therefore 82% of the VEP abnormalities demonstrated in the drug-exposed group were attributable to methadone exposure. A population attributable risk percent was not calculated as it is unlikely the comparison group recruited for this study were representative of the general population. In summary, drug-exposed infants had smaller amplitude VEP responses with fewer P1 components and abnormal / immature waveform morphology compared to matched comparison infants. Drug-exposed infants were over five times more likely to have an abnormal neonatal VEP compared to non-exposed infants.

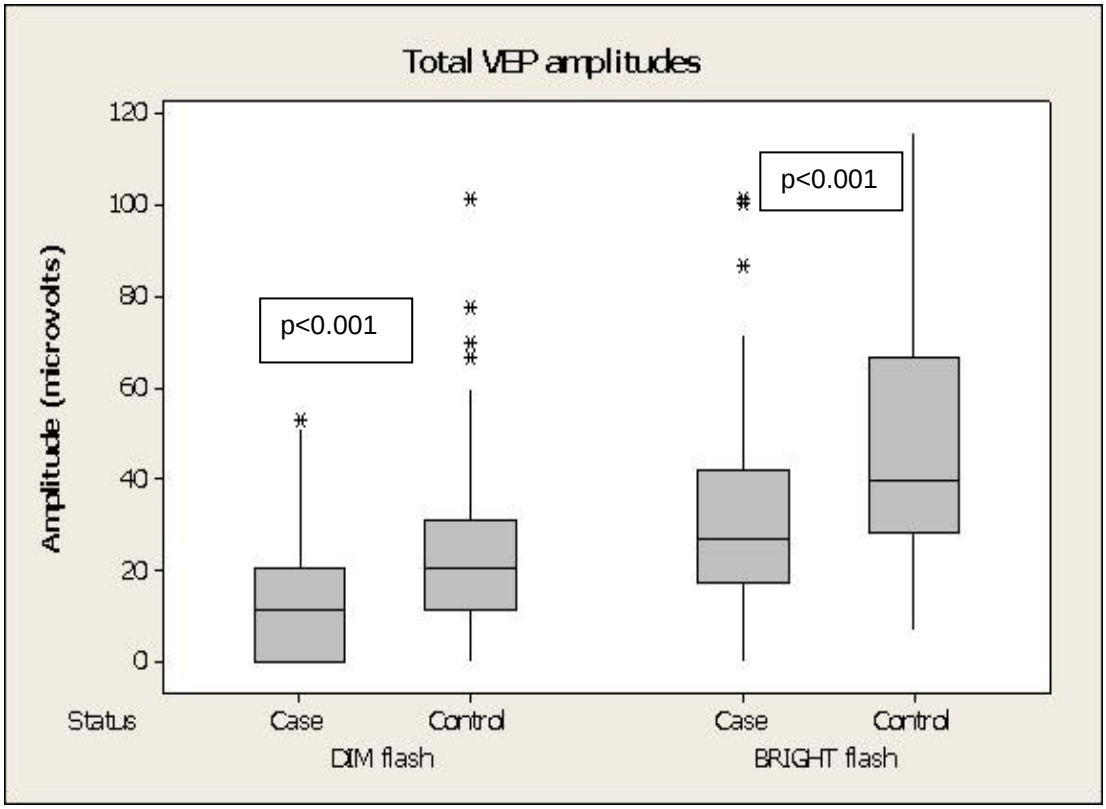


Figure 4-5 Boxplot of VEP amplitude in drug-exposed and comparison infants
The sum VEP amplitudes were significantly smaller in the drug-exposed infants compared to controls. This was statistically significant with both the dim and bright light stimuli.

Table 4-5 Neonatal flash VEPs for drug-exposed and comparison infants

	DIM FLASH			BRIGHT FLASH		
	Cases	*Controls*	*p-value*	*Cases*	*Controls*	*p-value*
P1 response	5%	10%	0.302	21%	48%	**0.001**
N2 response	11%	9%	0.244	38%	60%	**0.011**
P2 response	53%	86%	**<0.001**	89%	98%	**0.033**
N3 response	60%	60%	1.000	87%	84%	0.621
P1 latency (ms)	192 (137-211)	117 (107-145)	0.095	133 (118-175)	137 (114-157)	0.936
P2 latency (ms)	213 (198-239)	215 (205-215)	0.403	207 (191-221)	206 (191-228)	0.690
N3 latency(ms)	321 (285-358)	334 (310-353)	0.389	296 (247-329)	321 (250-357)	0.262

Data are given as percentage response (%) and median (inter-quartile range) for latencies. Percentage responses were compared using Chi-square tests and latencies compared using Mann-Whitney tests.

Morphology	Absent	Immature/atypical	Typical	Mature
Cases (n=100)	27%	32%	36%	5%
Controls (n=50)	6%	16%	68%	10%

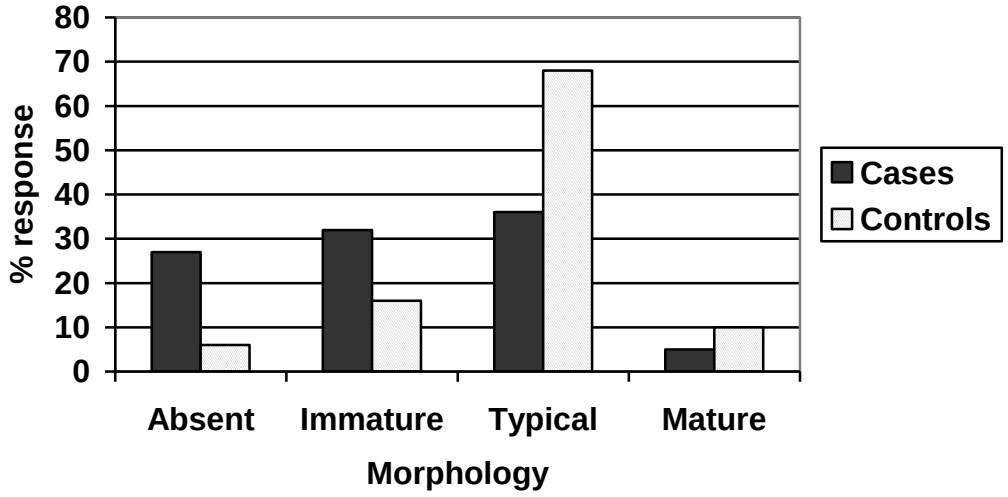


Figure 4-6 VEP morphology with dim flash stimulus.
The cases had more absent and immature responses and fewer typical and mature responses compared to controls: Chi ^{2}=19.1, p<0.001.

Morphology	Immature/abnormal	Typical	Mature
Cases (n=100)	28%	51%	21%
Controls (n=50)	10%	42%	48%

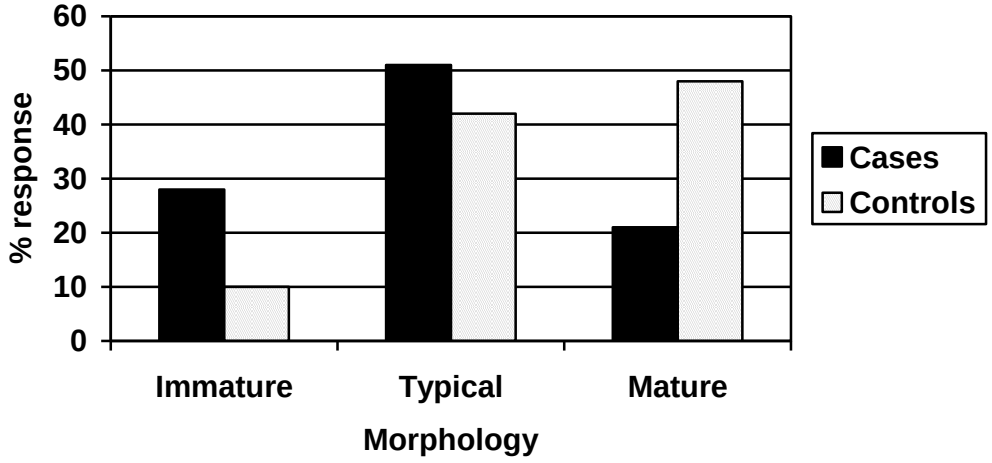


Figure 4-7 VEP morphology with bright flash stimulus
The cases had more immature/abnormal and fewer mature responses compared to controls: Chi ^{2} =13.6, p=0.001.

4.3.4 Investigation of potential confounders

Potential confounders were considered as variables which differed between the drug-exposed and comparison groups and which could have an independent effect on the newborn flash VEP. From the comparison demographics (Table 4-1), the infant OFC, maternal smoking status and proportion of mothers on prescribed antidepressants differed significantly between groups. Associations were therefore investigated between these variables and both VEP amplitude and latency.

There did not appear to be any association between VEP amplitude or latency and the infant OFC (VEP amplitude and OFC: Pearson's correlation coefficient= 0.11; p=0.193; VEP P2 latency and OFC: Pearson's correlation coefficient=-0.049; p=0.570). This was further illustrated by a scatter plot and linear regression analysis of the data (Figure 4-8).

The drug-exposed infants also had a higher proportion of mothers on prescribed antidepressant and/or antipsychotic medication compared to the control infants. To investigate any potential confounding effect of these maternal prescribed drugs on the newborn infant VEP, VEP parameters were compared between infants whose mothers were on medication and those not. The bright flash amplitude did not differ significantly between groups: median amplitude 23 μV antidepressant group *vs* 27.2 μV; Mann-Whitney test p = 0.345 (95% CI -5.2 to 13.6). There was no difference in bright flash P2 latency between groups: median P2 latency 205.5 ms antidepressant group *vs* 207 ms; Mann-Whitney test p=0.648. VEP morphology also did not differ (Chi ^{2} =0.56; p=0.761) between groups.

Mann-Whitney tests were also used to compare the VEP amplitudes and latencies of infants born to smoking mothers with infants born to non-smoking mothers. Infants of smoking mothers were found to have significantly smaller amplitude VEP responses compared to those of infants whose mothers did not smoke (median amplitude non-smokers 37.2μV *vs* median amplitude smokers 29.8μV; p=0.017; 95% CI 2.2 to 19.5) (Figure 4-9). The P2 latency did not significantly differ between groups (non-smokers 209ms *vs* smokers 207ms; p=0.363; 95% CI -7.01 to 20.00).

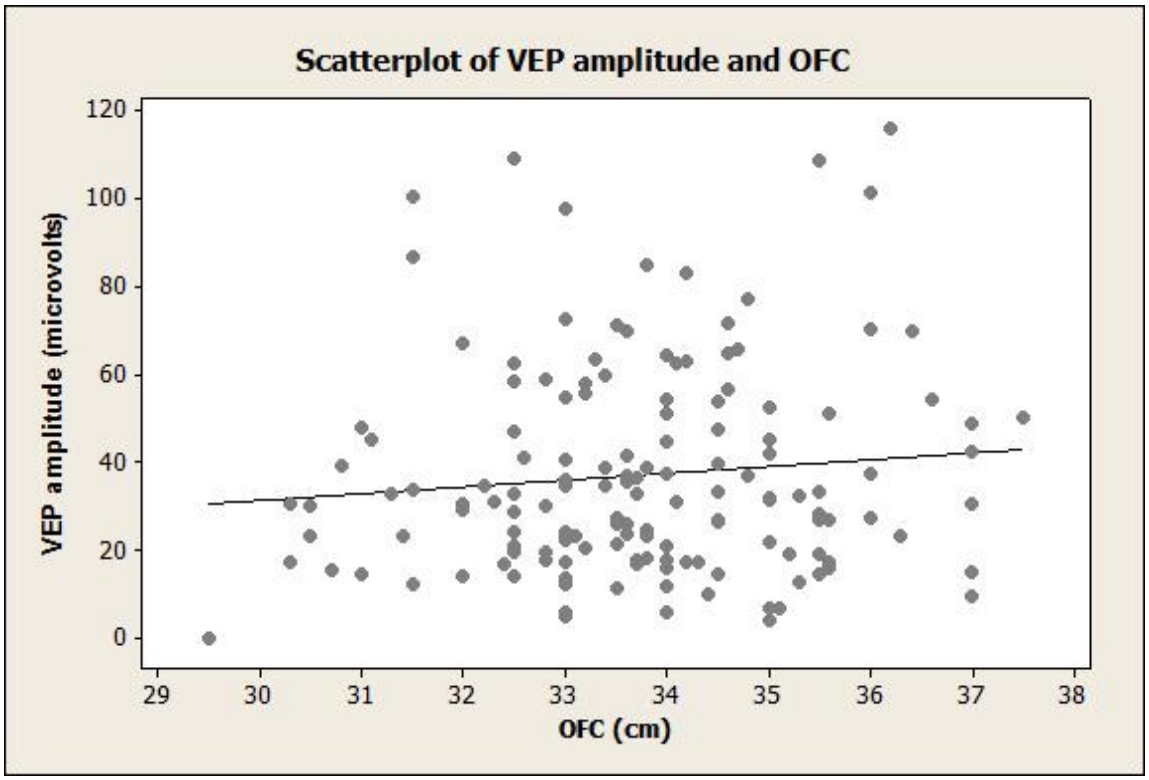


Figure 4-8 Scatterplot of VEP amplitude and OFC
There did not appear to be any significant correlation between VEP amplitude and infant OFC. Linear regression analysis: R^2 = 1.1%, p=0.19.

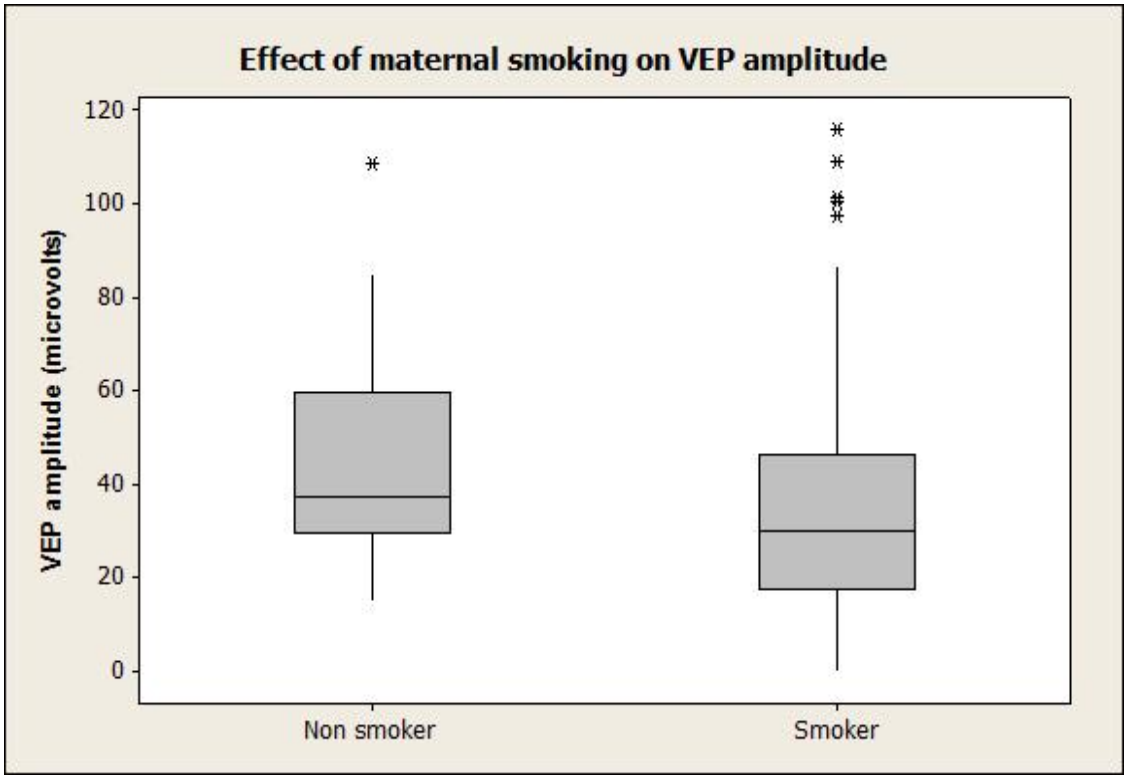


Figure 4-9 Boxplot of VEP amplitude and smoking status
VEP amplitudes were compared between infants born to smoking mothers and those born to non-smoking mothers. Infants born to smoking mothers had significantly smaller amplitude VEPs compared to non-smokers (Mann-Whitney test; p=0.017).

It was therefore necessary to use a linear regression model to assess if maternal smoking status was a potential confounder in the analysis of VEP amplitude and drug-exposure status. Both status group (case/control) and smoking status (smoker/non-smoker) were entered as predictor variables with VEP amplitude as the response variable as follows:

Linear Regression model: VEP amplitude with status group and smoking group

Predictor	Coef	SE Coef	T	P
Constant	48.519	4.574	10.61	0.000
Status gp	-14.714	4.337	-3.39	0.001
Smoking gp	-2.111	5.486	-0.38	0.701

Using this model, only status group was independently associated with VEP bright flash amplitude (p=0.001). Similarly, cases had significantly reduced VEP amplitude with the dim flash after correcting for maternal smoking (p=0.002).

Previous analysis had also demonstrated that the drug-exposed infants had significantly fewer P2 responses with the dim flash stimulus and fewer P1 and N2 responses with the bright flash stimulus compared to controls. Logistic regression analysis was used to correct for the potential confounding effect of smoking status on these binary outcomes.

Logistic Regression model: Bright P1 response with status group and smoking group

Predictor	Coef	SE Coef	Z	P
Constant	-0.024855	0.417291	-0.06	0.953
Status gp	-1.21275	0.414955	-2.92	0.003
Smoking gp	-0.092042	0.511556	-0.18	0.857

Using a logistic regression model, after correcting for smoking status, the drug-exposed infants were still significantly less likely to have a P1 response compared to control infants: p=0.003. Drug-exposed infants were also significantly less likely to have a bright flash N2 response than controls: p=0.008.

Logistic Regression model: Dim P2 response with status group and smoking group

Predictor	Coef	SE Coef	Z	P
Constant	2.42099	0.661865	3.66	0.000
Status gp	-1.44482	0.481440	-3.00	0.003
Smoking gp	-0.897456	0.694675	-1.29	0.196

After correcting for smoking status, the drug-exposed infants were significantly less likely to have a P2 response compared with control infants: p=0.003.

4.3.5 Relationship with NAS

A relationship between the neonatal flash VEP and NAS was investigated in two ways. Initially drug-exposed infants were classified as having NAS or not based on a requirement for pharmacological treatment. VEP parameters were then compared between the two groups. To further investigate possible differences between infants with varying severity of NAS, NAS was classified into four severity groups (1-4) as defined in the methods section (Chapter 3.10.3). Statistical tests were undertaken to compare any differences between these four groups.

Neonatal flash VEP parameters and their relationships to the presence of NAS are illustrated in Table 4-6. There was no difference in the presence of VEP components between infants who developed NAS and those who did not. Similarly, the latency of the VEP components did not differ between groups and the amplitude of the VEP responses did not significantly differ between those infants developing NAS and those not. The VEP morphology also did not differ between groups (dim flash stimulus: $Chi^2 = 1.18$, $p=0.875$; bright flash stimulus: $Chi^2 = 0.44$, $p=0.804$).

Table 4-6 Flash VEPs and NAS

	DIM			BRIGHT		
	No NAS	*NAS*	*p-value*	*No NAS*	*NAS*	*p-value*
P1 response	6%	4%	1.000	19%	23%	0.579
P2 response	55%	51%	0.715	89%	89%	0.913
N3 response	58%	62%	0.743	92%	81%	0.083
P1 latency (ms)	198	165	0.773	141	130	0.259
IQR	136-224	-		124-189	102-173	
P2 latency (ms)	216	208.5	0.837	208	203	0.122
IQR	198-240	195-242		192-240	190-217	
N3 latency (ms)	322	314	0.437	307	293	0.614
IQR	283-345	288-378		246-331	250-324	
Amplitude (μV)	10	12.4	0.653	27	28.8	0.771
IQR	0-20.4	0-27.8		17.1-38	17.1-44.5	

Data are percentage response (%), medians and inter-quartile ranges (IQR) for latencies and amplitude. Data are compared using Chi ^{2} tests for percentage responses (Fisher's exact test for dim P1 responses due to small numbers) and Mann-Whitney tests for latencies and amplitudes.

Kruskal-Wallis tests were used to investigate associations between the bright flash VEP parameters and the four NAS severity groups. There was no evidence of a difference between groups for VEP amplitude, P1 latency or P2 latency (Table 4-7). In addition the VEP morphology did not differ significantly between the four NAS groups ($Chi^2 = 2.79$, $p=0.815$).

In summary, there did not appear to be any evidence of a relationship between the neonatal flash VEP and the subsequent development or severity of NAS.

Table 4-7 Flash VEPs and NAS severity

Group	Amplitude (μV)	P1 latency (ms)	P2 latency (ms)
1) no NAS	29.8 (23.3-50.5)	133 (114-140)	206 (185-220)
2) mild NAS	23.2(13.9-35.4)	175 (127-191)	210 (195-254)
3) mod NAS	31.8 (16.1-47.2)	130 (100-173)	199 (190-216)
4) severe NAS	26.0 (18.2-44.5)	122 (107-177)	204 (187-223)
p-value	0.229	0.460	0.168

Data are median values (inter-quartile ranges). Kruskal-Wallis tests were used to compare data between groups.

4.3.6 Relationship with drug and alcohol exposure

A review of the case notes of all mothers and babies and a confidential maternal interview were undertaken. One hundred and thirty maternal urine samples were collected during pregnancy from 84 drug misusing women. Seventy infant urine samples were collected and 110 infant meconium samples (74 samples from drug exposed infants, 36 samples from controls). The results were collated as described in the methods section to provide a drug exposure status for each study infant.

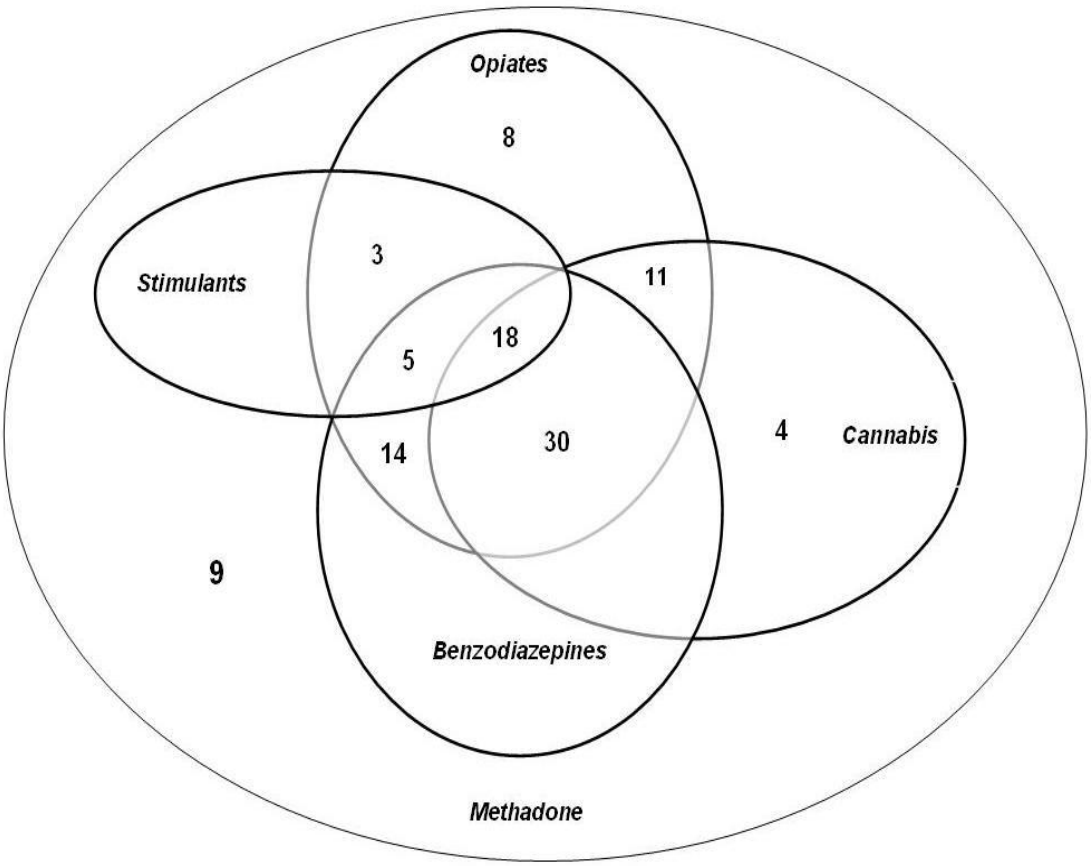
Drug exposure was compared between the different techniques of detection (maternal history, maternal urine toxicology, infant urine toxicology, infant meconium toxicology). Data from each of these four techniques were combined to provide a pattern of overall exposure for each infant and these data were used for classification into a drug exposure group. Drug exposure status is shown in Table 4-8. Meconium was more sensitive at detecting *in utero* drug exposure than postnatal infant urine for all the drugs of misuse investigated, and was also more sensitive than maternal history and maternal urine for most drugs.

Most infants were exposed to poly-drug misuse as illustrated in Figure 4-10. Only nine infants were exposed to methadone alone. A further eight infants were exposed to other opiates in addition to methadone, giving a subgroup of 17 neonates exposed to opiates alone. The most commonly misused drugs in addition to methadone were opiates (74%), benzodiazepines (66%) and cannabis (62%). Twenty six infants were exposed to stimulants (cocaine and/or amphetamines) in addition to other drugs. For the purpose of statistical analysis babies were classified into one of five drug exposure groups: group 1 = opiates alone (n=17), group 2 = opiates + cannabis (n=15), group 3 = opiates + benzodiazepines (n=14), group 4 = opiates + benzodiazepines + cannabis (n=30) and group 5 = other drug exposure including stimulants (n=26) (Figure 4-10).

Table 4-8 Drug exposure status

Drug	Mat History (n=102)	Maternal urine (n=84)	Infant urine (n=70)	Infant meconium (n=74)	Overall (n=102)
Methadone	100%	92%	61%	96%	**100%**
Opiate	54%	56%	36%	81%	**74%**
BDZ	51%	58%	33%	53%	**66%**
Amphetamine	2%	1%	0%	14%	**13%**
Cannabis	19%	39%	9%	65%	**62%**
Cocaine	5%	5%	3%	15%	**14%**

BDZ: benzodiazepine. Data are the percentage of positive results for each technique. The overall column combines the history and toxicology results to give a pattern of overall drug exposure.



Drug exposure group	Number of babies
1. Opiates only	17
2. Opiates + Cannabis	15
3. Opiates + Benzodiazepines	14
4. Opiates + Benzodiazepines + Cannabis	30
5. Other (stimulants)	26

Figure 4-10 Pattern of drug exposure in cases

Infants who had been exposed to stimulants *in utero* had significantly lower birth weights than those infants not exposed to stimulants: mean 2742 gm (SD 564) *vs* mean 2971 gm (SD 449), 2 sample t-test p=0.039. They also had smaller head circumferences but this was not statistically significant: mean 33.04 cm (SD 1.67) *vs* 33.67 cm (SD 1.47), 2 sample t-test p=0.072.

There was a positive correlation between NAS group and drug exposure group, suggesting that increased *in utero* drug exposure was associated with increased NAS severity (Pearson's correlation=0.243, p=0.014).

VEP amplitudes and latencies were compared between the five different drug exposure groups using Kruskal-Wallis tests as the amplitude and latency data were of skewed distribution. There were no significant differences in the VEP parameters between groups (Table 4-9, Figure 4-11). VEP morphology was also compared between groups using Chi-squared tests. For the purpose of statistical analysis, the typical and mature responses were classified together as normal responses and the absent, immature and atypical responses were classified together as abnormal responses. There were no differences in morphology between the five drug exposure groups (dim flash: Chi ^{2}= 1.019, p= 0.907; bright flash: Chi ^{2}= 4.150, p=0.386).

VEP parameters were also compared between the nine infants who had been exposed to methadone alone and the rest of the drug exposed cohort (n=91). VEP amplitude did not differ between groups: dim flash 12.6 μV *vs* 10.6 μV, Mann-Whitney test p=0.715; bright flash 23.7 μV *vs* 29.0 μV, Mann-Whitney test p=0.142. Neither did the proportion of P1 and P2 responses (Fisher's exact tests p=0.198 and p=0.167 respectively) and VEP morphology (Fisher's exact test p=0.707) differ between groups.

To further investigate the effects of different drugs of misuse on the neonatal VEP, regression analysis was undertaken. Drug exposure status for each of the drugs of misuse was entered as a predictor variable and VEP parameters entered as response variables (VEP amplitude in a linear regression model and P1 and P2 responses in binary logistic regression models). This allowed assessment of methadone exposure alone on VEP parameters after correcting for additional illicit drug use. Methadone exposed infants had significantly reduced amplitude

neonatal flash VEPs after correcting for other drug use (dim flash: p=0.009; bright flash: p=0.012). Methadone exposed infants were also significantly less likely to have a bright flash P1 response compared to control infants (p=0.001), less likely to have a bright flash N2 response (p=0.024) and less likely to have a dim flash P2 response (p=0.008) after correcting for additional illicit drug use.

Methadone dose

Associations were investigated between parameters of the neonatal flash VEP and the dose of prescribed maternal methadone prior to delivery. There were no significant correlations between maternal methadone dose and either VEP amplitude (Pearson correlation 0.060; p=0.561) or VEP P2 latency (Pearson correlation 0.108; p=0.327). In addition the VEP morphology did not differ between infants exposed to a high methadone dose *in utero* (>50mg) and those exposed to a lower dose (≤50mg): Chi^2=1.125; p=0.569. Similarly, the proportion of P1 and P2 responses did not differ between infants exposed to high versus lower methadone dose: P1 response Chi^2=0.549; p=0.460 and P2 response Chi^2=0.711; p=0.402.

In summary, there did not appear to be any differences in the VEPs between infants in different drug exposure groups and regression analysis suggested the difference in VEP parameters between drug-exposed and control infants was associated with methadone exposure and not the other drugs of misuse.

Excess alcohol exposure

Excess alcohol exposure *in utero* was defined as infants who had elevated FAEEs in meconium ≥ 10,000 nanograms/gram. Meconium samples from 84 infants were analysed for the presence of FAEEs (63 cases and 21 controls); the remainder of the samples were insufficient for analysis. Twenty six drug exposed infants (26/63 tested, 41%) and five comparison infants (5/21 tested, 23%) were exposed to excess alcohol *in utero*. None of these infants had a clinical diagnosis of fetal alcohol syndrome.

VEP parameters were compared between infants who had been exposed to excess alcohol *in utero* and those who had not. There was no difference in

median VEP amplitude (excess alcohol group = 30.6 μV *vs* no excess alcohol group = 31.7 μV, Mann-Whitney test p = 0.827) or VEP morphology (Chi ^{2} = 0.195, p = 0.907) between groups. The proportion of P1 and P2 components also did not differ between groups (Chi ^{2} = 0.094, p = 0.759 and Chi ^{2} = 0.124, p = 0.725 respectively).

Regression models were used to correct for the effect of excess alcohol exposure *in utero* on the neonatal flash VEP. Excess alcohol exposure was entered as a predictor variable along with drug exposure status (case/control) in a linear regression model for VEP amplitude and binary logistic regression models for P1 and P2 responses. Drug-exposed infants had smaller amplitude VEPs after correcting for alcohol excess (bright flash amplitude p<0.001, dim flash amplitude p<0.001): excess alcohol exposure had no independent effect on the neonatal flash VEP amplitude (p=0.664). Drug-exposed infants were also less likely to have a bright flash P1 response (p=0.001) and less likely to have a dim flash P2 response (p<0.001) after correcting for excess alcohol exposure.

Table 4-9 Flash VEPs and drug exposure

	Drug exposure group					
	1	**2**	**3**	**4**	**5**	**p-value**
Dim amp (μV)	14.3	8.7	10.8	9.3	12.7	0.544
IQR	(7.4-30.5)	(0-16.9)	(0-26.0)	(0-19.4)	(1.8-34.3)	
Bright amp (μV)	26.1	31.0	31.5	25.2	23.9	0.706
IQR	(18.0-45.9)	(17.1-38.8)	(22.0-51.3)	(16.0-39.3)	(16.7-46.9)	
P1 lat (ms)	130	-	140	145	127	0.858
IQR	-	-	(126-181)	(107-188)	(102-175)	
P2 lat (ms)	194	200	208	204	218	0.122
IQR	(177-207)	(180-233)	(194-215)	(196-215)	(197-241)	
N3 lat (ms)	279	319	323	269	307	0.219
IQR	(232-306)	(262-324)	(250-381)	(237-314)	(269-332)	

Data are medians. Inter-quartile ranges are given below in brackets. Kruskal-Wallis tests were used for comparisons between the five drug exposure groups.

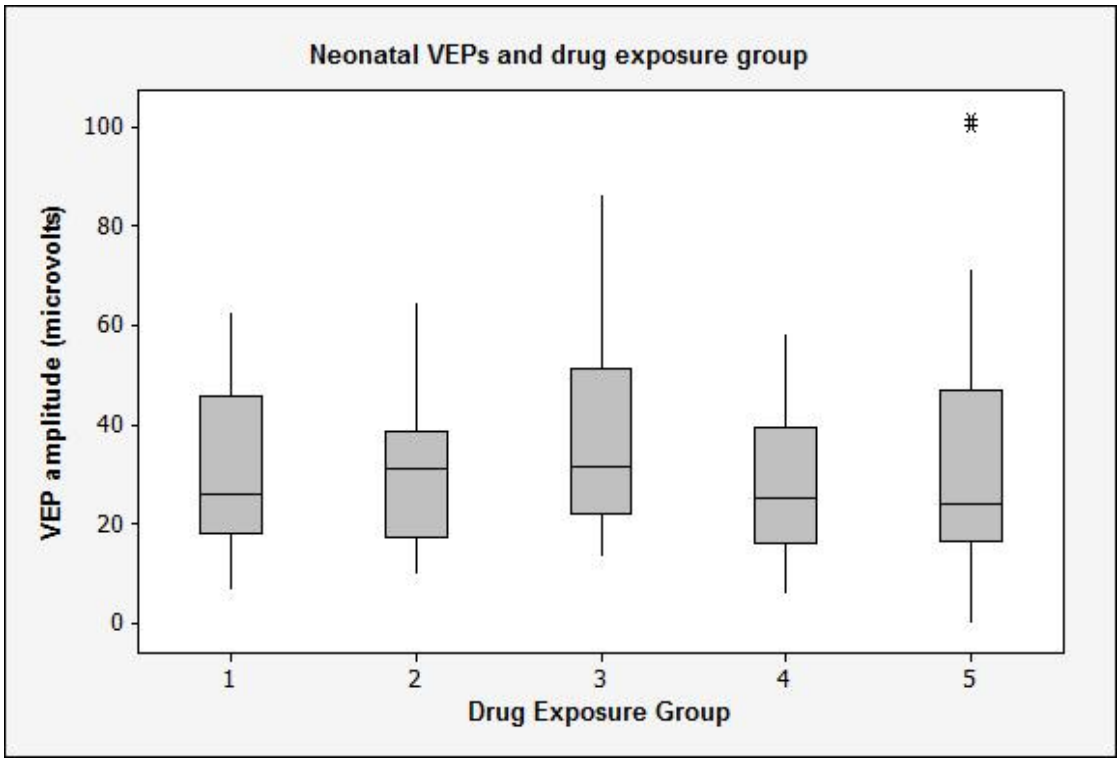


Figure 4-11 Boxplot of VEP amplitudes and drug exposure
Group 1: opiates alone, group 2: opiates + cannabis, group 3: opiates + benzodiazepines, group 4: opiates + benzodiazepines + cannabis, group 5: other drug exposure including stimulants. There were no differences in VEP amplitude between groups: Kruskal-Wallis test, p= 0.706.

4.4 Neonatal flicker VEPs

Six sets of flicker data were lost due to a computer system failure and therefore 144 sets of flicker VEPs were available for analysis (99 drug-exposed infants and 45 comparison infants).

4.4.1 Flicker responses

Responses were defined as a signal to noise ratio (SNR) > 2.8 at the stimulus frequency and were investigated at F1, F2 and F3 (fundamental response and harmonic responses). Similar to the pilot work, the largest proportion of responses at F1 was obtained with the 4.64 Hz stimulus for both drug-exposed and comparison infants (Table 4-10). The proportion of responses present reduced with increasing frequency of the stimulus.

The proportion of F1 responses at each frequency was compared between drug-exposed infants and comparison infants for both the pulse wave and sine wave stimuli. There was little difference between the groups although with the 5.86 Hz sine wave stimulus more drug-exposed infants had a response than comparisons (62.6% cases *vs* 42.2% controls, p=0.021; 95% CI for difference 0.03, 0.38) (Table 4-10). Using a Bonferroni correction to account for the number of statistical tests performed however, a p-value of < 0.005 would be considered significant. A multivariate, repeated measures logistic regression model was performed to test the overall difference between groups after correcting for wave type and frequency. Using this model a p-value of 0.345 was obtained suggesting there was no overall difference in the proportion of F1 responses between drug-exposed infants and comparisons.

There was no difference in the proportion of F2 responses between groups at any of the stimulus frequencies using either sine or pulse wave (Table 4-11). A multivariate, repeated measures logistic regression model was used to compare overall F2 responses between cases and controls and produced a p-value of 0.653.

As it is possible to have a harmonic response in the absence of a detectable fundamental response, the response status was further classified as a positive

response at any of F1, F2 or F3. Using this definition, the proportion of responses increased with over 90% of comparison infants having a detectable response with the 4.64 Hz sine wave stimulus (Table 4-12). Using this classification, there were still few differences between groups with respect to response rate. The cases had a higher proportion of responses with the 7.32 Hz sine stimulus compared to controls (66.7% *vs* 48.9%, p=0.044) (Table 4-12). However, a multivariate repeated measures, logistic regression model applied to investigate the overall difference between groups found no statistically significant difference between cases and controls (p=0.572).

In summary, there were no significant differences in the proportion of flicker VEP responses between drug-exposed and comparison infants.

Table 4-10 Proportion of F1 responses

	PULSE			SINE		
Freq (Hz)	*Case*	*Control*	*p-value*	*Case*	*Control*	*p-value*
4.64	51/99	24/45	0.859	70/99	31/45	0.846
	(51.5%)	(53.3%)	(-0.19, 0.16)	(70.7%)	(68.9%)	(-0.14, 0.18)
5.86	37/99	15/45	0.636	62/99	19/45	0.021
	(37.4%)	(33.3%)	(-0.13, 0.21)	(62.6%)	(42.2%)	(0.03, 0.38)
7.32	37/99	13/45	0.308	53/99	20/45	0.309
	(37.4%)	(28.9%)	(-0.08, 0.25)	(53.5%)	(44.4%)	(-0.08, 0.27)
12.7	14/99	11/45	0.158	15/99	7/45	0.950
	(14.1%)	(24.4%)	(-0.25, 0.04)	(15.2%)	(15.6%)	(-0.13, 0.12)
18.55	14/99	10/45	0.256	17/99	5/45	0.315
	(14.1%)	(22.2%)	(-0.22, 0.06)	(17.2%)	(11.1%)	(-0.06, 0.18)

Data are the number of significant F1 responses as defined by SNR > 2.8. The percentage response is given in brackets below. Statistical analysis was done using Z tests for 2-proportions. The 95% confidence interval for the difference is given in brackets below the p-value. A multivariate, repeated measures, logistic regression model was applied to test the overall difference between cases and controls and found no significant difference between groups (p=0.345).

Table 4-11 Proportion of F2 responses

	PULSE			SINE		
Freq (Hz)	*Cases*	*Controls*	*p-value*	*Cases*	*Controls*	*p-value*
4.64	34/99	16/45	0.888	61/99	31/45	0.390
	(34.3%)	(35.6%)	(-0.18, 0.16)	(61.6%)	(68.9%)	(-0.24, 0.09)
5.86	24/99	6/45	0.101	43/99	18/45	0.698
	(24.2%)	(13.3%)	(-0.02, 0.24)	(43.4%)	(40.0%)	(-0.14, 0.21)
7.32	15/99	8/45	0.697	18/99	6/45	0.447
	(15.2%)	(17.8%)	(-0.16, 0.11)	(18.2%)	(13.3%)	(-0.08, 0.17)
12.7	12/99	4/45	0.547	7/99	3/45	0.929
	(12.1%)	(8.9%)	(-0.07, 0.14)	(7.1%)	(6.7%)	(-0.08, 0.09)
18.55	15/99	5/45	0.494	7/99	5/45	0.450
	(15.2%)	(11.1%)	(-0.08, 0.16)	(7.1%)	(11.1%)	(-0.15, 0.06)

Data are the number of significant F2 responses as defined by SNR > 2.8. The percentage response is given in brackets below. Statistical analysis was done using Z tests for 2-proportions. The 95% confidence interval for the difference is given in brackets below the p-value. A multivariate, repeated measures, logistic regression model was applied to test the overall difference between cases and controls and found no significant difference between groups (p=0.653).

Table 4-12 Proportion of F1, F2 or F3 responses

	PULSE			SINE		
Freq (Hz)	*Cases*	*Controls*	*p-value*	*Cases*	*Controls*	*p-value*
4.64	73/99	36/45	0.399	86/99	42/45	0.199
	(73.7%)	(80%)	(-0.21, 0.08)	(86.9%)	(93.3%)	(-0.16, 0.03)
5.86	62/99	24/46	0.296	81/99	32/45	0.169
	(62.6%)	(53.3%)	(-0.08, 0.27)	(81.8%)	(71.1%)	(-0.05, 0.26)
7.32	55/99	22/45	0.457	66/99	22/45	0.044
	(55.6%)	(48.9%)	(-0.11, 0.24)	(66.7%)	(48.9%)	(0.005, 0.35)
12.7	34/99	17/45	0.692	28/99	12/45	0.840
	(34.3%)	(37.8%)	(-0.20, 0.14)	(28.3%)	(26.7%)	(-0.14, 0.17)
18.55	31/99	18/45	0.316	28/99	14/45	0.732
	(31.3%)	(40.0%)	(-0.26, 0.08)	(28.3%)	(31.1%)	(-0.19, 0.13)

Data are the number of significant responses at F1, F2 or F3 (as defined by SNR > 2.8). The percentage response is given in brackets below. Statistical analysis was done using Z tests for 2-proportions. The 95% confidence interval for the difference is given in brackets below the p-value. A multivariate, repeated measures, logistic regression model was applied to test the overall difference between cases and controls and found no significant difference between groups (p=0.572).

4.4.2 Flicker amplitudes

Flicker VEP F1 amplitudes were investigated between drug-exposed and comparison infants. An Anderson-Darling test for normality demonstrated that flicker VEP amplitude data were not normally distributed and data were therefore described as medians and inter-quartile ranges.

Table 4-13 shows the median flicker VEP amplitudes for all infants. The drug-exposed infants had larger median amplitude responses at all frequencies tested, which was statistically significant at 4.64 Hz sine, 5.86 Hz sine, 7.32 Hz sine, 12.7 Hz pulse and 18.55 Hz sine. The maximum amplitude difference between groups was 0.7 μV. The optimal stimulus frequency was 4.64 Hz for both cases and controls. A multivariate, repeated measures analysis of variance model was performed to test the overall difference between groups after correcting for the stimulus frequency and wave form. Due to the skewed distribution of the flicker amplitude data, the data was logarithm transformed for this statistical model. This produced a p-value of <0.001 suggesting that the cases had overall significantly larger amplitude flicker responses compared to controls.

Flicker F2 amplitudes were also compared between groups (Table 4-14). Again there was a trend for the drug-exposed infants to have larger amplitude flicker responses but using a multivariate, repeated measures analysis of variance model, there was no statistically significant overall difference between groups (p=0.090).

In summary, the proportion of flicker responses did not differ between drug-exposed infants and comparison infants, and the optimum stimulus frequency was identical for both groups (4.64 Hz). The drug-exposed infants had increased amplitude F1 responses at all frequencies which reached statistical significance.

Table 4-13 F1 amplitude

	PULSE			SINE		
Freq (Hz)	*Cases*	*Controls*	*p-value*	*Cases*	*Controls*	*p-value*
4.64	2.46	2.16	0.313	3.28	2.5	0.049
	(1.56-3.26)	(1.39-3.08)	(-0.21, 0.68)	(2.23-5.06)	(1.71-4.01)	(0.001,1.21)
5.86	1.45	1.26	0.111	2.09	1.57	0.005
	(1.15-1.89)	(0.62-2.20)	(-0.06,0.54)	(1.42-2.99)	(1.18-2.14)	(0.17, 0,87)
7.32	1.54	1.20	0.176	1.84	1.43	0.016
	(0.89-2.14)	(0.81-1.87)	(-0.09,0.46)	(1.27-2.41)	(0.94-2.01)	(0.06,0.62)
12.7	0.86	0.71	0.028	0.74	0.72	0.734
	(0.62-1.18)	(0.45-0.97)	(0.02,0.32)	(0.54-1.02)	(0.53-1.09)	(-0.10,0.15)
18.55	0.61	0.54	0.526	0.59	0.47	0.029
	(0.39-0.84)	(0.41-0.79)	(-0.07,0.15)	(0.42-0.90)	(0.35-0.73)	(0.01,0.22)

Data are medians with inter-quartile ranges given below in brackets. Mann-Whitney tests were used for statistical analysis. The 95% confidence interval for difference is given below the p-value in brackets. A multivariate, repeated measures analysis of variance model was applied to test the overall difference between cases and controls and found the cases to have significantly larger amplitude F1 flicker amplitudes ($p<0.001$).

Table 4-14 F2 amplitude

	PULSE			SINE		
Freq (Hz)	*Cases*	*Controls*	*p-value*	*Cases*	*Controls*	*p-value*
4.64	0.72	0.69	0.990	1.32	1.19	0.786
	(0.39-1.06)	(0.38-1.15)	(-0.16,0.17)	(0.82-1.95)	(0.84-1.97)	(-0.25,0.31)
5.86	0.57	0.49	0.022	0.80	0.65	0.098
	(0.37-0.86)	(0.27-0.49)	(0.02,0.25)	(0.49-1.13)	(0.42-1.00)	(-0.23,0.27)
7.32	0.36	0.39	0.983	0.46	0.43	0.904
	(0.27-0.57)	(0.20-0.61)	(-0.09,0.08)	(0.24-0.66)	(0.23-0.69)	(-0.08,0.09)
12.7	0.24	0.23	0.786	0.22	0.20	0.359
	(0.15-0.33)	(0.15-0.36)	(-0.06,0.04)	(0.13-0.38)	(0.13-0.34)	(-0.03,0.07)
18.55	0.19	0.14	0.084	0.18	0.15	0.313
	(0.11-0.26)	(0.10-0.24)	(-0.006,0.07)	(0.11-0.26)	(0.10-0.24)	(-0.02,0.06)

Data are medians with inter-quartile ranges given below in brackets. Mann-Whitney tests were used for statistical analysis. The 95% confidence interval for difference is given below the p-value in brackets. A multivariate, repeated measures analysis of variance model was applied to test the overall difference between cases and controls and found no significant difference between groups (p=0.090).

4.4.3 Neonatal flicker VEPs and NAS

The flicker F1 amplitude was compared between infants who developed NAS (defined as requiring pharmacological treatment) and infants who did not develop NAS.

There was little difference in the flicker F1 amplitude between groups (Table 4-15). As there were few other differences in the flicker VEP between cases and controls, further investigative statistics relating to the development of NAS were not undertaken.

Table 4-15 Flicker amplitudes and NAS

	PULSE			SINE		
Freq (Hz)	*No NAS*	*NAS*	*p-value*	*No NAS*	*NAS*	*p-value*
4.64	2.41	2.45	0.369	3.36	3.13	0.761
	(1.75-3.3)	(1.47-3.1)	(-0.31,0.77)	(1.98-4.67)	(2.23-5.15)	(-0.91,0.69)
5.86	1.59	1.36	0.049	2.25	1.99	0.214
	(1.23-2.16)	(1.13-1.65)	(0.007,0.48)	(1.58-3.03)	(1.39-2.99)	(-0.17,0.75)
7.32	1.56	1.57	0.621	1.72	1.76	0.401
	(0.82-2.16)	(0.97-2.01)	(-0.41,0.29)	(1.28-2.23)	(1.21-2.66)	(-0.49,0.20)
12.7	0.83	0.94	0.117	0.73	0.75	0.722
	(0.49-1.17)	(0.71-1.22)	(-0.32,0.04)	(0.53-1.01)	(0.54-1.03)	(-0.17,0.12)
18.55	0.52	0.67	0.270	0.55	0.62	0.148
	(0.37-0.83)	(0.40-0.85)	(-0.23,0.07)	(0.37-0.77)	(0.48-0.91)	(-0.20,0.04)

Data are medians with inter-quartile ranges given below in brackets. Mann-Whitney tests were used for statistical analysis. The 95% confidence interval for difference is given below the p-value in brackets

5 Chapter 5 Results: Follow up data

One hundred and seven infants were reviewed at six months: 81 of 102 (79%) drug-exposed infants and 26 of 50 (52%) comparison infants recruited. Overall study retention was 70%. Reasons for non attendance (45 infants) included: parent/carer uncontactable (25 infants: 56%), did not attend a pre-arranged appointment on two or more occasions (11 infants: 24%), geographically unable to attend (three infants: 7%) and declined follow up participation (six infants: 13%). All infants attending for follow up completed both clinical visual and developmental assessment.

5.1 Growth parameters and general health

Median age at six month assessment was 27 weeks (IQR 26-28) for both groups (no significant difference; Mann-Whitney test p=0.231). There were no significant differences between groups in terms of weight or OFC at six month follow up: mean weight cases 7.52 kg (SD 1.05) *vs* mean weight controls 7.94 kg (SD 1.09) , 2-sample t test p=0.110; mean OFC cases 43.26 cm (SD 1.54) *vs* mean OFC controls 43.83 cm (SD 1.88), 2-sample t test p=0.146.

In addition, in view of the relatively high drop-out rate of comparison infants, the demographic characteristics of comparison infants who were followed up were compared to those of comparison infants who were not followed up. There were no significant differences in birth weight (2 sample t-test p=0.445), OFC (2 sample t-test p=0.712, gestation (Mann-Whitney test p=0.984), 5-minute Apgar score (Mann-Whitney test p=0.263) or DEPCAT score (Mann-Whitney test p=0.258) between groups.

Fourteen of the drug-exposed cohort had been admitted to hospital following discharge (17%). Reasons for admission were: bronchiolitis (five infants), gastro-oesophageal reflux disease (two infants), non-specific viral illness (two infants), hernia repair (three infants), urinary tract infection (one infant) and pyloric stenosis (one infant). Three of the comparison infants (12%) had been admitted

to hospital: two with a non-specific viral illness and one with viral meningitis. All infants had been commenced on a weaning diet when seen for follow up and no infants were breast fed at six months of age. All infants were on term commercial formula milk.

All comparison infants were in the care of their parents. Sixty-one drug exposed infants were in the care of their parents, 14 infants were in foster care (17%) and six infants were accommodated with a family member (7%). Overall 24% of the drug exposed cohort was accommodated at six months of age.

5.2 Infant Pattern VEPs

Pattern onset VEPs were recorded in 105/107 infants at the six month assessment (79 cases, 26 controls). One infant did not undergo testing due to a computer system failure and one infant was too unsettled for recording.

Ninety two infants underwent 120 minute check size VEP recording (67 cases, 25 controls), 103 infants underwent 60 minute check size recording (77 cases, 26 controls) and 94 infants underwent 15 minute check size recording (70 cases, 24 controls). 40 infants underwent 60 minute pattern reversal VEP recording (26 cases, 14 controls).

5.2.1 Pattern VEP responses

Comparison infants

All comparison infants tested had reproducible VEP responses at all three check sizes: 25/25 responses at 120 minute check size, 26/26 responses at 60 minute check size, 24/24 responses at 15 minute check size.

Drug- exposed infants

All cases tested (67/67) had responses present at the large (120 minute) check size. Three drug-exposed infants had an absent VEP response at the medium (60 minute) check size and only 51/70 drug-exposed infants had a response at the small (15 minute) check size. Compared to controls, drug-exposed infants were

significantly less likely to have a pattern VEP response present at the small check size; Fisher's exact test p=0.003.

5.2.2 Pattern VEP latencies

VEP latency data were of a skewed distribution and were therefore described as medians and inter-quartile ranges. Statistical tests were done using Mann-Whitney tests.

Amplitudes and latencies were compared between drug-exposed infants and comparisons for the C2 peak response (C2a) at each check size. The cases had significantly delayed latencies at the 120 minute and 15 minute check sizes (Table 5-1, Figure 5-1). At the 60 minute check size the cases had delayed latency C2 responses but this did not quite reach statistical significance using a non-parametric test (p=0.063 with Mann-Whitney test). When 60 minute C2 latency data were logarithm transformed to a normal distribution and tested with a 2-sample t test, the p-value was 0.050. C1 latency data did not differ significantly between groups although the numbers of C1 responses were low.

Table 5-1 Pattern VEP latencies

Check size	C2 latency (ms)		p-value
	Cases	**Controls**	
120 minute	115.0	99.0	0.019
	(94-136)	(93-142)	
60 minute	110.0	106.0	0.063 **(0.050*)**
	(96-126)	(94-112)	
15 minute	122.0	108.0	0.028
	(109-130)	(92-125)	

Data are medians with inter-quartile ranges given in brackets below. Statistical tests were done using Mann-Whitney tests and **(*)** 2-sample t test for logarithm transformed 60 minute data.

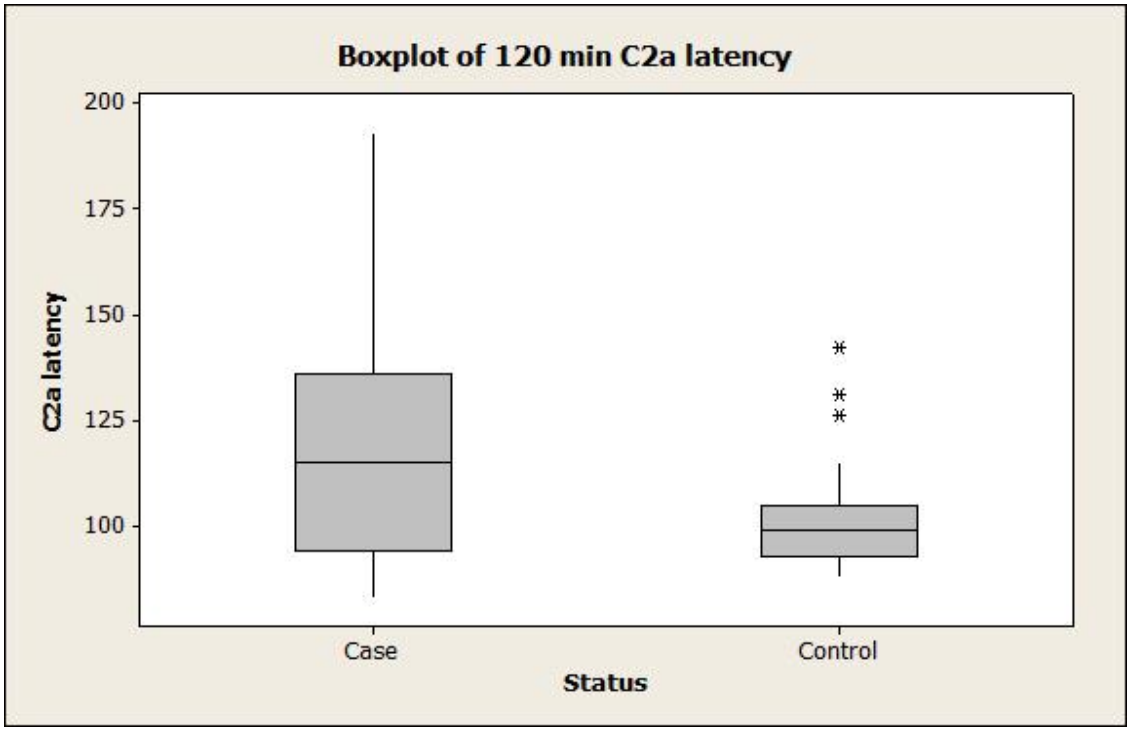


Figure 5-1 Boxplot of pattern VEP latency
The drug exposed infants had delayed latency C2 peak responses at the large (120 minute) check size: median latency 115 ms cases *vs* 99 ms controls; Mann-Whitney test p=0.019.

5.2.3 Pattern VEP amplitudes

Pattern VEP amplitude data were of a skewed distribution and were therefore described as medians and inter-quartile ranges. The drug-exposed infants had significantly reduced amplitude responses at the 60 minute check size and a trend to reduced amplitude responses at the other check sizes (Table 5-2, Figure 5-2).

Table 5-2 Pattern VEP amplitudes

Check size	C2 amplitude (μV)		p-value
	Cases	**Controls**	
120 minute	24.0	26.0	0.091
	(15-33)	(17-43)	
60 minute	25.0	34.0	**0.005**
	(19-34)	(28-39)	
15 minute	13.0	17.0	0.191
	(10-20)	(10-25)	

Data are medians with inter-quartile ranges given in brackets below. Statistical tests were done with Mann-Whitney tests.

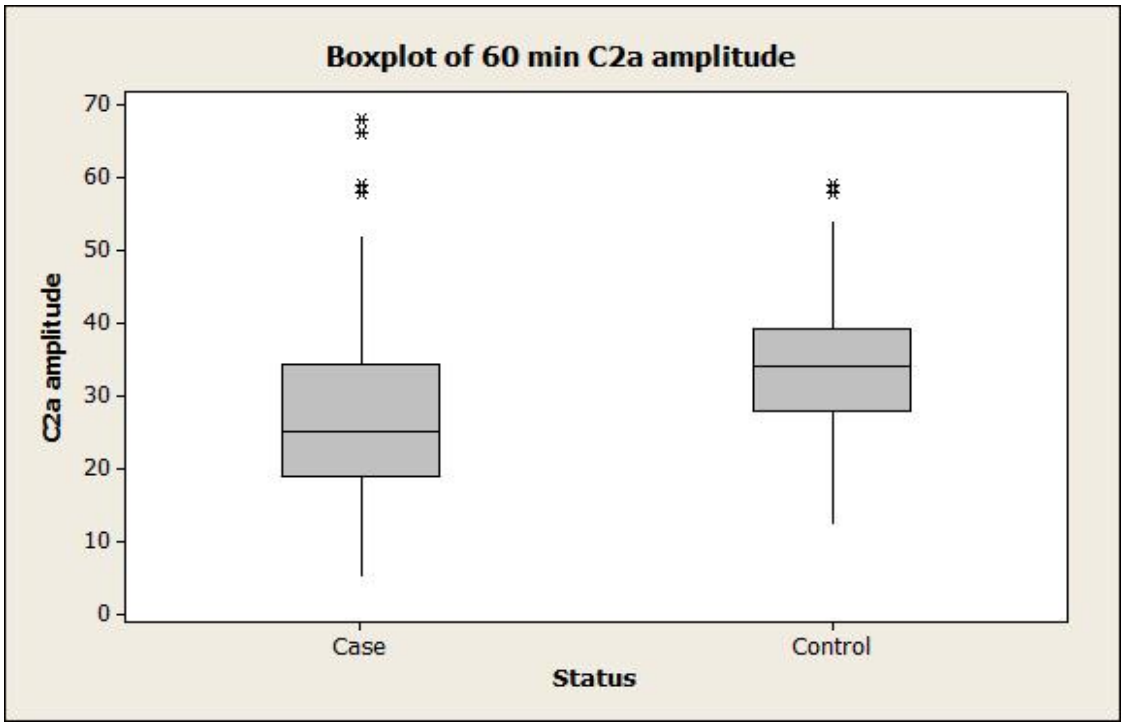


Figure 5-2 Boxplot of pattern VEP amplitude
The drug exposed infants had smaller amplitude responses at the medium (60 minute) check size; median amplitude 25 μV cases *vs* 34 μV controls; Mann-Whitney test p=0.005.

The comparison infant pattern VEPs were used to define limits of normality to enable drug-exposed infant pattern VEPs to be classified as normal or abnormal. An absent VEP response at any check size was classified as abnormal. The upper limit values for VEP latency and lower limit values for VEP amplitude were used as cut off values for normality and are demonstrated below:

Check size	Latency upper limit (ms)	Amplitude lower limit (μV)
120 minute	142	8
60 minute	127	12
15minute	157	2

Using these definitions 34/79 (43%) drug-exposed infants had an abnormal pattern VEP: cases 34/79 abnormal pattern VEPs *vs* controls 0/26 abnormal pattern VEPs; Fisher's exact test $p<0.001$.

5.2.4 Pattern VEPs and NAS

C2 amplitudes and latencies were compared between drug exposed infants who developed NAS (defined as requiring pharmacological treatment) and those who did not. There were no significant differences or trends in the VEP parameters between groups, suggesting that NAS and/or its pharmacological treatment does not account for the changes demonstrated in visual electrophysiology (Table 5-3).

Table 5-3 Pattern VEP parameters and NAS

	NAS	No NAS	p-value
120 minute			
C2 latency (ms)	105.0 (92.0-139.0)	126.0 (100.3-135.7)	0.525
C2 amplitude (μV)	20.0 (14.0-28.0)	25.0 (18.5-35.5)	0.095
60 minute			
C2 latency (ms)	106.0 (95.0-125.0)	118.0 (98.0-127.0)	0.410
C2 amplitude (μV)	26.0 (20.0-37.0)	23.0 (17.0-31.0)	0.392
15 minute			
C2 latency (ms)	122.5 (116.0-130.5)	118.0 (98.5-130.5)	0.412
C2 amplitude (μV)	13.5 (10.0-23.3)	13.0 (8.0-17.5)	0.199

Data are medians with inter-quartile ranges given below in brackets. Statistical tests were done using Mann-Whitney tests.

5.2.5 Pattern VEPs and drug exposure

Pattern VEP parameters were compared between infants in the five different drug exposure groups. There were no significant differences between groups suggesting the effect shown was secondary to opiate exposure and not other substances of misuse (Table 5-4).

Table 5-4 Pattern VEP parameters and drug exposure

VEP parameter	Drug exposure group					
	1	2	3	4	5	p-value
120 minute						
C2 lat (ms)	115.0	132.5	132.0	101.0	113.5	0.356
	(97-135)	(105-146)	(97-142)	(91-131)	(92-138)	
C2 amp (μV)	26.5	18.0	24.0	20.0	22.5	0.330
	(24-35)	(12-30)	(14-32)	(14-28)	(17-39)	
60 minute						
C2 lat (ms)	104.5	119.5	98.0	110.5	114.5	0.613
	(97-127)	(104-134)	(94-123)	(97-125)	(94-130)	
C2 amp (μV)	22.0	31.5	25.0	22.0	28.5	0.340
	(18-28)	(20-37)	(19-45)	(15-32)	(20-35)	
15 minute						
C2 lat (ms)	123.0	121.0	122.0	118.0	126.0	0.787
	(88-132)	(103-162)	(111-124)	(116-131)	(115-131)	
C2 amp (μV)	15.0	9.5	12.0	13.0	14.5	0.429
	(11-20)	(8-19)	(7-13)	(9-19)	(10-22)	

Data are medians with inter-quartile ranges given in brackets below. Statistics were done using Kruskall-Wallis tests. C2 lat: latency, C2 amp: amplitude.

5.2.6 Pattern reversal VEPs

Forty infants underwent pattern reversal VEP recording (26 cases and 14 controls). All comparison infants had a pattern reversal response present (14/14). Four drug-exposed infants had an absent response; two of these infants had nystagmus present. These two infants had recordable 60 min pattern onset VEPs supporting the evidence that pattern onset VEPs are more reliable in infants with nystagmus compared to pattern reversal (106).

There was a trend to delayed VEP latencies in the drug-exposed group but this was not significant: median P100 latency cases (n=22) 113 ms *vs* median latency controls (n=14) 107 ms; Mann-Whitney test p = 0.167 and median N75 latency cases (n=13) 76 ms *vs* median latency controls (n=7) 73 ms; Mann-Whitney test p = 0.381.

5.3 Visual outcomes

All infants underwent a clinical visual assessment. A fail was defined as the presence of strabismus, nystagmus, delayed visual maturation, reduced visual acuity or a refractive error which was confirmed by ophthalmology on cycloplegic refraction and required correction with glasses. Visual acuity (VA) from the comparison group was used to define normal acuity for this age group: the poorest VA amongst the control infants was 6/48 (one infant), and so this was defined as the limit of normality. Thus reduced VA was poorer than 6/48. A borderline assessment was defined as 1) minor visual abnormalities, 2) refractive error which was not confirmed by ophthalmology due to failure to attend following referral, or 3) moderate refractive error confirmed by ophthalmology, but not prescribed glasses and remaining under follow up.

Overall 40% of drug-exposed infants failed the visual test battery (32 infants). A further nine infants (11%) were described as borderline: these were infants with possible refractive errors not assessed by ophthalmology (two infants), moderate refractive errors not prescribed glasses (three infants), exophoria (two infants), anisocoria (one infant) and absent blink response (one infant). 2/26 (8%) of the comparison group failed the visual assessment: one infant had an intermittent strabismus (esotropia) and one had a refractive error (myopia). Significantly more drug-exposed infants failed the visual test battery than comparison infants: 32/81 cases failed *vs* 2/26 controls failed; Fisher's exact test p=0.003.

Clinical visual outcomes are summarised as follows:

	Abnormal vision	Normal vision
Methadone exposed	32	49
Non-exposed	2	24

The relative risk of an abnormal visual assessment was:

RR= 32/(32+49) / 2/(2+24) = 5.1.

Therefore infants exposed to methadone *in utero* were five times more likely to have an abnormal visual assessment than matched infants not exposed to methadone.

The attributable risk percent was also calculated to estimate the proportion of disease amongst the exposed group which was attributable to methadone exposure.

% AR = incidence in exposed group-incidence in non exposed group/ incidence in exposed group x 100 = 80%.

Therefore 80% of the visual abnormalities demonstrated in the drug-exposed group were attributable to methadone exposure. A population attributable risk percent was not calculated as it is unlikely the comparison group recruited for this study were representative of the general population.

Nystagmus was present in nine of the drug-exposed cohort (11%). No control infant demonstrated nystagmus. The nystagmus was horizontal in all cases and varied in being manifest (four infants), latent (three infants) and manifest latent (one infant). Of the nine infants with nystagmus, six had been treated for NAS in the neonatal period (66%) and eight (89%) were known to have been exposed to benzodiazepines in addition to opiates *in utero*. There was no significant difference in the incidence of NAS between infants with nystagmus and without nystagmus: 6/9 (66%) infants with nystagmus *vs* 35/72 (49%) infants without nystagmus; Fisher's exact test p=0.347. There was also no significant difference in the incidence of benzodiazepine exposure between infants with nystagmus and without nystagmus: 8/9 (89%) infants with nystagmus *vs* 49/72 (68%) infants without nystagmus; Fisher's exact test p=0.268.

Strabismus was detected in 20 drug-exposed infants (25%). 12 infants had an exotropia and eight infants had an esotropia.

Reduced VA (defined as VA poorer than 6/48) was detected in 18 drug-exposed infants (22%). The reduced acuity was associated with other ophthalmic abnormalities in 11 of these infants. Delayed visual maturation was diagnosed in infants who had reduced VA or visual inattentiveness, but who subsequently showed catch up in visual development: 11 infants (14%) demonstrated DVM.

Forty nine drug exposed infants were seen by ophthalmology in addition to having a six month study assessment. Age at ophthalmology assessment varied from eight months to 14 months. Discrepancies between study visual assessment and ophthalmology assessment were: five infants had a strabismus noted at the study assessment and no strabismus when seen by ophthalmology; four infants had strabismus noted by ophthalmology which had not been present when seen for the study assessment.

Visual outcomes were compared between infants who had NAS in the neonatal period (defined as requiring pharmacological treatment) and infants who did not have NAS. There was no significant association between six month visual outcome and NAS: Chi ^{2} test; p=0.239.

	NAS	No NAS
Pass	21	28
Fail	20	12

Visual outcomes were also compared between infants in the five different drug exposure groups. There was no significant association between six month visual outcome and drug exposure group: Chi ^{2} test; p=0.528.

	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5
Pass	9	9	6	14	11
Fail	3	3	5	11	10

Binary logistic regression was performed to account for the potential confounding effect of excess alcohol exposure on six month visual outcome. Excess alcohol was defined as significantly elevated FAEEs on meconium analysis as described in the methods section. After correcting for excess alcohol exposure, drug-exposed infants were significantly less likely to pass the six month visual assessment (p=0.007). There was also no significant difference in the proportion of infants failing the six month visual assessment between infants exposed to excess alcohol *in utero* and those not exposed: Chi ^{2} = 0.035; p=0.852. There was no independent effect of *in utero* alcohol exposure on visual outcome (p=0.790).

Visual outcomes were compared between infants exposed to high dose methadone (>50mg) and those exposed to lower dose methadone (≤50mg) to assess for a dose-response relationship. Although a greater proportion of infants exposed to the higher dose failed the visual assessment compared to the lower dose (65% versus 44%), this did not quite reach statistical significance: Chi-squared test p=0.055.

In summary, drug-exposed infants were at significantly greater risk of visual abnormalities at six months of age than non drug-exposed matched infants after correcting for excess alcohol exposure *in utero*. There was no significant association between six month visual outcome and NAS or drug exposure group suggesting that all drug exposed infants were at risk.

5.4 Developmental outcomes

All infants underwent a full Griffiths developmental assessment. A fail was defined as a GQ < 85: all of these infants were referred for further assessment to either hospital or community follow up clinics. All comparison infants passed the developmental assessment; in contrast eight drug-exposed infants failed (26/26 controls pass *vs* 73/81 cases pass; Z test for 2-proportions, p=0.003).

The GQ and sub-quotient scores were not normally distributed; data were therefore described as medians and comparisons done with Mann-Whitney tests. Developmental outcomes for each sub-quotient and overall GQ scores are shown in Table 5-5. The drug-exposed infants had significantly reduced development quotients for all sub scales and reduced GQ scores compared to comparison infants (Figure 5-3). Two drug exposed infants had abnormalities of tone: one infant had generalised hypotonia and one had unequal tone of the upper limbs.

Potential confounders for developmental outcome were maternal smoking, antidepressant use and excess alcohol intake during pregnancy. A linear regression model was used to assess if these factors confounded the developmental outcome as follows:

Regression equation
GQ = 106 - 8.06 Status no - 2.18 Smoker + 0.05 Antidepressant - 1.17 Alcohol

Predictor	Coef SE	Coef	T	P
Constant	105.873	1.791	59.10	0.000
Status	-8.056	2.024	-3.98	0.000
Smoker	-2.184	2.283	-0.96	0.341
Antidepressant	0.052	2.600	0.02	0.984
Alcohol	-1.165	1.722	-0.68	0.500

Therefore, the drug-exposed infants had significantly poorer developmental GQ scores after correcting for maternal smoking status, antidepressant use and excessive alcohol intake during pregnancy. Adjusted p-values after correcting for maternal smoking status, antidepressant use and excessive alcohol intake in pregnancy are shown in Table 5-5.

Of the eight drug-exposed infants who failed the developmental assessment, six infants (75%) had concurrent significant visual problems which included reduced visual acuity in all cases. Four of the eight infants who failed the developmental assessment had abnormal pattern VEPs (absent response to 60 minute check size in three, delayed response to 15 minute check size in one).

Infants who failed the visual assessment performed poorer on their developmental scales than infants who passed the visual assessment: median GQ infants who failed = 95 *vs* median GQ infants who passed = 100; Mann-Whitney test $p < 0.001$. A linear regression model was used to assess the independent effect of visual impairment on developmental outcome (outcome variable: GQ score, predictor variables: group, smoking status, alcohol excess status, visual impairment). Using this model, visual impairment was found to be independently associated with lower developmental scores ($p<0.001$).

Developmental sub-quotients and GQ scores were compared between drug-exposed infants who received pharmacological treatment for NAS and those who did not. Infants treated for NAS performed significantly poorer in their locomotor, personal-social and language subscales, as well as having a significantly reduced overall GQ score (Table 5-6).

Developmental outcomes were also compared between infants who were accommodated (n=20) and infants who were in the care of their biological parents (n=61). Accommodated infants performed poorer on their developmental scales than infants not accommodated: median GQ accommodated infants = 92 *vs* median GQ not accommodated = 97; Mann-Whitney test $p = 0.003$. However, infants who were accommodated were more likely to have been treated for NAS in the newborn period (accommodated infants 70% NAS *vs* non accommodated 43%; Chi ^{2} test $p = 0.039$) and were more likely to have been exposed to benzodiazepines and stimulants *in utero* (accommodated infants 90% poly-drug exposure *vs* non accommodated 63%; Chi ^{2} test $p = 0.015$).

Table 5-5 Developmental outcome of cases and controls

Development	Cases (n=81)	Controls (n=26)	p-value	Adjusted p-value
Locomotor	102 (93-107)	111 (101-111)	<0.001	0.006
Personal-social	94 (88-96)	99 (94-103)	<0.001	0.001
Language-hearing	105 (105-109)	109 (105-109)	<0.001	0.007
Eye-hand	94 (86-99)	104 (99-104)	<0.001	0.001
Performance	96 (86-100)	101 (101-111)	<0.001	0.002
GQ	**97 (93-100)**	**105 (101-108)**	**<0.001**	**<0.001**

Data are medians (inter-quartile ranges). Statistical analysis was done using Mann-Whitney tests. Adjusted p-values are after correcting for maternal smoking status, antidepressant use and excess alcohol consumption in pregnancy using linear regression models.

Table 5-6 Developmental outcome and NAS

Development	NAS (n=40)	No NAS (n=41)	p-value
Locomotor	98 (87-102)	102 (94-107)	0.012
Personal-social	89 (84-94)	94 (89-99)	0.016
Language-hearing	105 (100-105)	105 (105-109)	0.011
Eye-hand	94 (84-99)	94 (90-99)	0.137
Performance	96 (86-100)	96 (91-101)	0.329
GQ	**95 (91-99)**	**99 (94-102)**	**0.008**

Data are medians (inter-quartile ranges). Statistical analysis was done using Mann-Whitney tests.

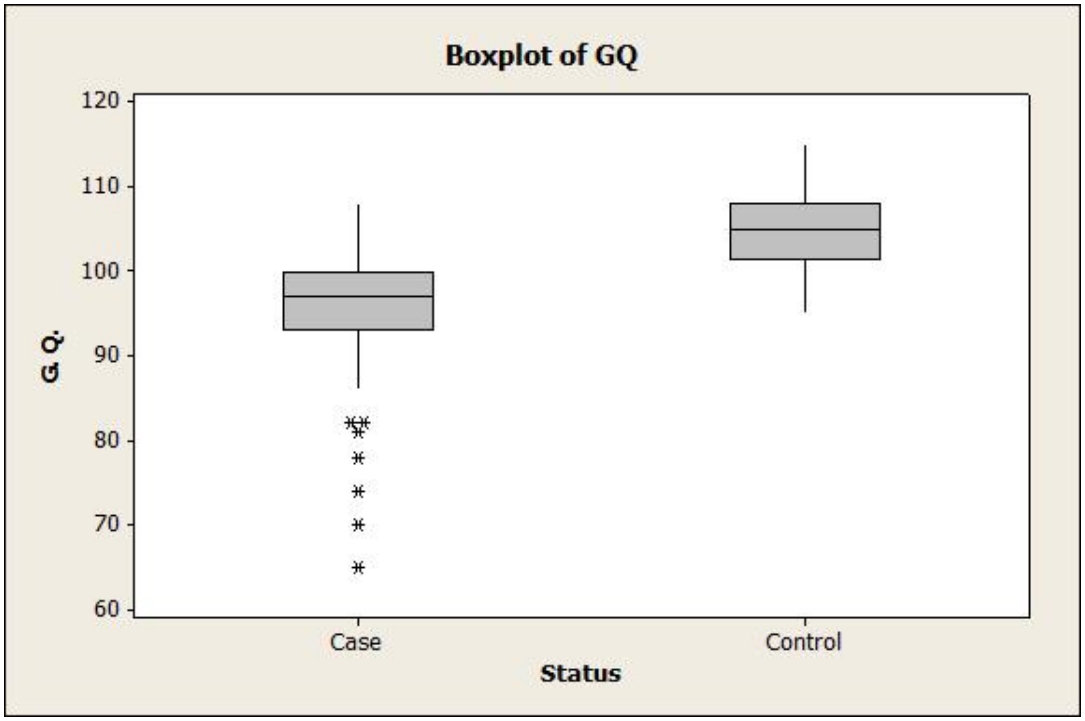


Figure 5-3 Boxplot of Griffiths GQ scores
Drug-exposed infants had significantly reduced overall neurodevelopment scores compared to control infants: Mann-Whitney test p<0.001. Six of the eight infants who failed the developmental assessment (GQ < 85) had coexisting visual impairment.

5.4.1 Development and drug exposure group

Developmental quotients were compared between infants in the five different drug exposure groups. There were significant differences between groups with infants exposed to increasing *in utero* poly drug exposure having lower development quotients (Table 5-7, Figure 5-4).

An association was sought between the dose of prescribed maternal methadone prior to delivery and six month neurodevelopmental outcomes. The median methadone dose did not differ between infants who passed and failed the developmental assessment: median dose of infants who failed = 52.5 *vs* median dose of infants who passed = 50.0; Mann-Whitney test p = 0.923. In addition there was no correlation between prescribed maternal methadone dose and Griffiths GQ score: Pearson's correlation p = 0.175.

Table 5-7 Developmental outcome and drug exposure

Development	Drug exposure group					
	1	2	3	4	5	p-value
Locomotor	104.5 (98-118)	102.0 (95-107)	102.0 (98-107)	98.0 (93-102)	93.0 (84-102)	0.020
Personal-social	96.5 (94-103)	94.0 (90-98)	94.0 (89-94)	89.0 (84-94)	89.0 (84-97)	0.062
Language	107.0 (101-109)	109.0 (105-109)	105.0 (105-109)	105.0 (105-105)	105.0 (98-105)	0.115
Eye-hand	94.0 (94-99)	99.0 (94-104)	94.0 (89-99)	94.0 (82-99)	94.0 (84-97)	0.039
Performance	96.0 (91-101)	96.0 (91-101)	96.0 (91-101)	96.0 (86-96)	91.0 (82-96)	0.092
GQ	**100** (97-103)	**99.5** (98-103)	**99.0** (94-100)	**95.0** (93-97)	**93.0** (89-98)	**0.002**

Data are medians with inter-quartile ranges given in brackets below. Statistical tests were done using Kruskal-Wallis tests.

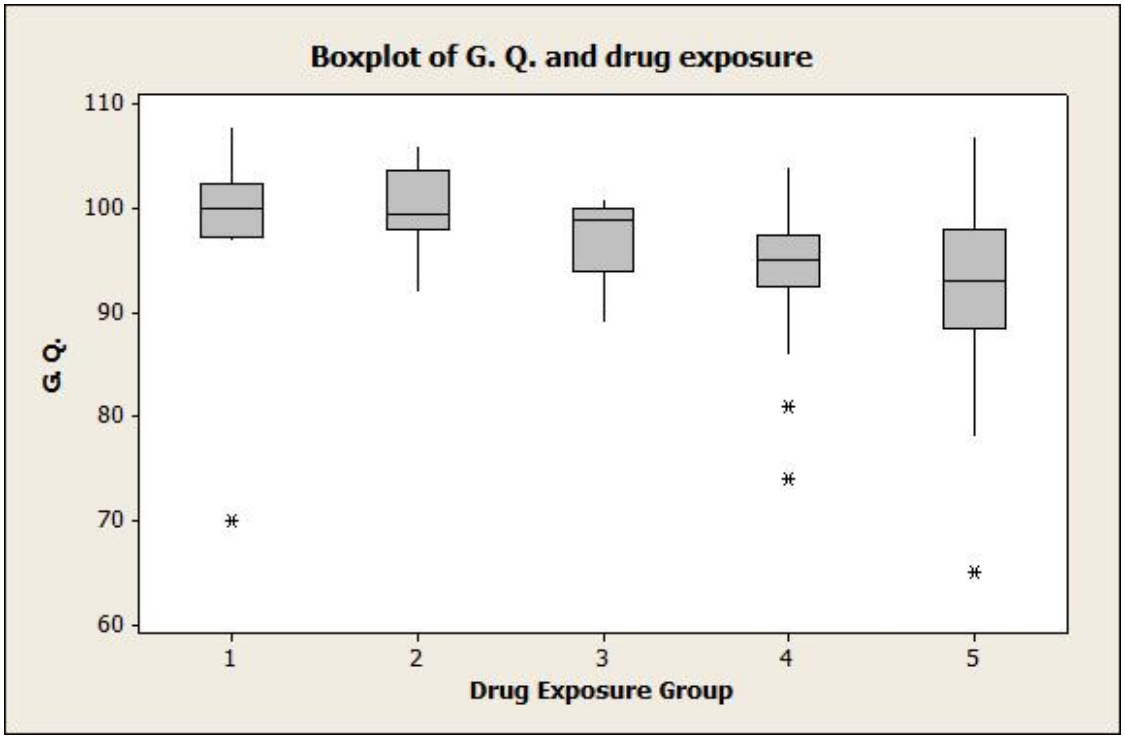


Figure 5-4 Boxplot of development scores and drug exposure
There were significant differences between groups with infants exposed to increasing poly drug misuse having lower scores (Kruskal-Wallis test; p=0.002).

In summary, at six months of age drug-exposed infants had lower neurodevelopmental scores compared to comparison infants matched for gestation, birth weight and socio-economic group even after correcting for maternal smoking status, antidepressant use and excess alcohol intake during pregnancy. Infants who were treated for NAS performed poorer than infants not treated for NAS and infants exposed to poly drug misuse *in utero* performed poorer than infants exposed to opiates alone.

5.5 Neonatal VEPs and outcomes

5.5.1 Neonatal flash VEPs and visual outcome

Seventy nine drug-exposed infants underwent both neonatal flash VEP recording and six month clinical visual assessment. Neonatal flash VEPs were compared between infants who passed and failed the clinical visual assessment at six months of age. For the purpose of analysis infants in the borderline category were defined as normal. There were no differences in morphology (Chi ^{2} test; p=0.329), presence of P1 components (Chi ^{2} test; p=0.596) or presence of P2 components (Chi ^{2} test; p=0.466) between groups. Median VEP amplitude did not differ between groups with either the bright flash (pass 29 μV *vs* fail 22.4 μV; Mann-Whitney test p=0.159) or the dim flash (pass 11 μV *vs* fail 9.6 μv; Mann-Whitney test p=0.927). Similarly, median P1 and P2 latencies did not differ between groups: P1 latency pass 130 ms *vs* fail 149 ms, Mann-Whitney test p=0.751; P2 latency pass 207 ms *vs* fail 207 ms, Mann-Whitney test p=0.547.

The association between the neonatal flash VEP and six month clinical visual outcome is shown in Table 5-8. Neonatal flash VEPs were classified as normal / abnormal as previously described (Chapter 4.3.3). The sensitivity of an abnormal neonatal VEP at detecting an abnormal visual outcome was 60% (18/ 18+12). Specificity was 51% (25/ 24+25). Positive and negative predictive values of the neonatal flash VEP were 43% and 68% respectively.

Table 5-8 Neonatal flash VEPs and visual outcome

		Visual outcome	
		Abnormal (Fail)	Normal (Pass)
Neonatal flash VEP	Abnormal	18	24
	Normal	12	25

5.5.2 Neonatal flash VEPs and developmental outcome

Seventy nine drug-exposed infants underwent both neonatal flash VEP recording and developmental assessment at six months of age. An abnormal developmental assessment was defined as a GQ score < 85. Flash VEPs were defined as normal / abnormal as previously described (Chapter 4.3.3). The relationship between the neonatal VEP and subsequent developmental outcome is shown in Table 5-9. An abnormal neonatal flash VEP had a high sensitivity (5/ (5+1) = 83%) but low specificity (36/ (36+37) = 49%) for predicting developmental outcome. The predictive value for abnormal developmental outcome of an abnormal neonatal flash VEP (positive predictive value) was 12% (5/ 37+5) and the predictive value for normal developmental outcome of a normal neonatal flash VEP was 97% (36/ 36+1).

Table 5-9 Neonatal flash VEPs and developmental outcome

		Developmental outcome	
		Abnormal (Fail)	Normal (Pass)
Neonatal flash VEP	Abnormal	5	37
	Normal	1	36

5.5.3 Neonatal flash VEPs and six month pattern VEPs

Seventy seven drug-exposed infants underwent both neonatal flash VEP recording and six month pattern VEP recording. Flash and pattern VEPs were designated as normal / abnormal as previously described. Table 5-10 demonstrates the association between the newborn flash VEP and six month pattern follow up VEP. There was little correlation between the newborn flash VEP and the six month pattern VEP: the sensitivity and specificity of an abnormal neonatal flash VEP at predicting an abnormal pattern VEP at 6 months were 39% and 39% respectively (positive predictive value = 33% and negative predictive value = 46%).

Table 5-10 Neonatal flash VEPs and infant pattern VEPs

		Six month pattern VEP	
		Normal	Abnormal
Neonatal flash VEP	Normal	17	20
	Abnormal	27	13

5.6 Summary of six month assessment

At six months of age methadone-exposed infants were more likely to have visual abnormalities than comparison infants, even after correcting for excess *in utero* alcohol exposure (40% *vs* 8%; adjusted p=0.007). The attributable risk was 80% suggesting that 80% of visual abnormalities seen in the drug-exposed cohort were attributable to methadone exposure. Abnormalities in the methadone-exposed cohort included nystagmus (11%), strabismus (25%) and reduced VA (22%).

Electrophysiological abnormalities persisted at six months of age: methadone-exposed infants had smaller amplitude pattern VEPs (25 μV *vs* 34 μV; p=0.005) with delayed peak latencies (115ms *vs* 99ms; p=0.019) and fewer responses at the small check size (p=0.003), compared to controls.

Methadone-exposed infants had significantly lower neurodevelopmental scores compared to comparison infants (GQ 97 for cases *vs* 105 for controls; p<0.001), even after correcting for maternal smoking, antidepressant treatment and excess alcohol consumption during pregnancy. Infants exposed to poly-drug misuse and treated for NAS in the newborn period performed particularly poorly on their neurodevelopmental scores. Visual impairment was independently associated with poor neurodevelopmental outcome.

The neonatal VEP had a low positive predictive value for six month visual and neurodevelopmental outcome and would therefore seem to be of limited value in predicting which infants warrant follow-up assessment.

6 Chapter 6 Discussion

This study was prompted by awareness that a growing number of patients referred to the local paediatric ophthalmology and visual electrophysiology departments with visual problems had a history of methadone exposure, and by increasing evidence of opiate related visual problems, as outlined in Chapter 1. Substitute methadone is the currently recommended treatment for pregnant opiate-dependent women, with advantages for mother and baby, including stabilisation of maternal lifestyle and reduced incidence of IUGR (5,129). The disadvantages of methadone use during pregnancy include an increased incidence of NAS and possible detrimental effects on infant visual and neurological development. The latter are variably reported in the literature, and have not been properly quantified to date. This study sought to explore the effects of *in utero* methadone exposure upon early infant visual development and also to assess the use of the VEP in predicting infant outcomes including NAS and subsequent visual and neurological development.

6.1 Subject demographic characteristics

Mothers:

Women who misuse drugs in pregnancy commonly suffer other consequences of social deprivation such as physical and mental ill health and poor nutritional status. The vast majority of drug-misusing mothers in this study were from the lower socioeconomic groups (median DEPCAT 7). They did however have a normal BMI (median 23), and this did not differ significantly from that of the comparison mothers. Cigarette smoking was more common in the drug-misusing population than the population as a whole: 95% of drug-misusing mothers in the study cohort smoked compared to 60% of the comparison mothers. It is recognised that self reporting significantly underestimates the number of pregnant smokers and therefore the incidence of smoking in both groups may have been higher than reported (130). Mental health problems requiring pharmacological treatment were present in 14% of drug-misusing mothers – this is comparable to the 12% described in a large local cohort of drug-misusing mothers by Dryden *et al* (8).

Infants:

It is well recognised that infants of drug-misusing mothers are more likely to be born prematurely and to suffer IUGR compared to infants of non-drug-misusing mothers (10,11). It was therefore very important to match drug exposed infants with comparison infants for gestation and birth weight, and this objective was achieved. As anticipated, the mean birth weight of the drug-exposed cohort was below the 50th centile (2892 grams; 25th centile). Despite matching for birth weight, the drug-exposed infants had significantly smaller head sizes compared to comparisons. This effect appeared to be due to the higher proportion of smoking mothers in the drug-exposed group: after correcting for smoking status there was no longer any significant difference in head sizes. This finding is in contrast to those of Shipton *et al* who found significantly smaller head circumferences in a much larger cohort of methadone exposed infants compared to gestation and social deprivation matched non-methadone exposed infants of smoking mothers (131). Breast feeding rates were similarly poor in both groups, denying infants the many advantages of breast feeding.

Drug exposure:

In common with other authors, and consistent with the pilot data, a high incidence of poly-drug misuse was found in the study cohort (8,32,122). The most commonly misused substances were illicit opiates and benzodiazepines. Exposure to poly pharmacy makes interpretation of study results complicated and necessitated the recruitment of a large number of infants. The sample size calculation estimated that 100 infants would require to be recruited to provide a cohort of 20 infants exposed to methadone alone, but in reality only nine infants in the drug-exposed cohort were exposed to methadone alone. It is likely that the comprehensive collection of toxicology samples, including infant meconium, provided more accurate information than was available in the pilot study. The vast majority of women on the methadone programme continued to use illicit opiates during pregnancy and it should be noted that that there is evidence that the beneficial effects of substitute methadone on infant birth weight are lost with concurrent illicit opiate use (14). Identifying the substances used by the mother is crucial if appropriate recommendations are to be made about substitute treatment.

Alcohol exposure:

Meconium analysis was undertaken to assess for excess alcohol consumption in pregnancy. Overall 41% of the drug-exposed cohort and 23% of comparisons tested had elevated FAEEs on meconium analysis suggestive of heavy alcohol intake during pregnancy although no infant had a clinical diagnosis of fetal alcohol syndrome. Regression models were used to correct for the potential confounding effect of excess alcohol exposure on both visual and developmental outcomes: this has not previously been documented in the published literature.

Neonatal abstinence syndrome:

Forty eight percent of the drug-exposed cohort received treatment for NAS; which is comparable with the 40-80% reported in other studies and consistent with previous local audit (4,8). First line treatment was oral morphine solution and second line treatment was phenobarbital (30), similar to practice in other units in the UK (132). Predictably infants requiring treatment for NAS had a significantly longer hospital stay than infants not treated, but even untreated infants stayed in hospital three times longer than comparisons. These data underline the significant resources which these infants utilise within the health care setting.

6.2 Neonatal visual electrophysiology

6.2.1 Neonatal VEPs

In the neonatal period, there were significant differences between the flash VEPs of drug-exposed infants and those of matched comparison infants. The most common flash VEP abnormalities detected in the drug-exposed cohort were reduced amplitudes, immature waveforms and an absence of P1 components. In comparison the control infants' VEPs were of larger amplitude and of mature waveform with a greater proportion of both P1 and P2 components. Infants exposed to methadone *in utero* were over five times more likely to have an abnormal neonatal VEP than non-exposed infants (relative risk of 5.6).The pilot study finding of delayed VEP latencies in drug-exposed infants was not replicated

in the larger study: this may reflect better matching of gestational age between cases and controls.

Prenatal substance misuse appears to result in an immaturity in the evolution of normal cortical visual pathways: this electrophysiological finding accords with the clinical finding of delayed visual maturation described in Pilot Study 4 and by other authors (64,125). All drug exposed infants' neonatal VEPs were recorded prior to commencement of oral morphine or phenobarbital treatment, so this was not a confounding factor.

There was no relationship between the neonatal VEP and the onset or severity of NAS, suggesting that the electrophysiological abnormalities described in association with maternal drug misuse were not reflective of the temporary cortical upset caused by neonatal withdrawal. This is an important negative finding, as infants with no NAS who tend to be discharged early from hospital with no follow up demonstrate the same abnormalities in their neonatal visual electrophysiology as those infants kept under close follow up.

Similarly, there were no differences in VEPs between infants in different sub-groups of illicit drug exposure. Regression analysis was used to investigate further the independent effects of different drugs of misuse on the newborn VEP and suggested that the differences in flash VEPs demonstrated between drug-exposed and comparison infants were associated with *in utero* methadone exposure and not the other drugs of misuse. It is possible that the other drugs of misuse may have had an independent effect on the newborn infant VEP but the sub-groups were too small to provide sufficient power to detect a difference. It is also possible that any differences were masked by the effect of methadone exposure. As all the study infants had been exposed to methadone *in utero* we were unable to determine the effects of other illicit drug use alone on the infant VEP.

A change in the flash luminance resulted in significant differences in flash VEPs with a brighter light stimulus producing larger amplitude responses with reduced latencies and more mature waveform morphology. This highlights the importance of standardising flash stimuli for research trials. Data are not

comparable between laboratories unless equipment has been calibrated to exactly the same standards.

In contrast to the flash VEP, there were only minor differences in flicker VEPs between drug-exposed and comparison infants. The flicker VEP is a novel technique and little normative data are available for the newborn period. Studies suggest that the optimal flicker frequency increases with increasing postnatal age and maturity; however the significance of the flicker VEP amplitude is not yet understood. It is therefore not possible to explain the differences demonstrated in the flicker VEP amplitude, which although of statistical significance, may not be of clinical significance.

6.2.2 Proposed mechanism for alteration in VEPs

The VEP is a cortically generated visual response and therefore any adverse effect of opiates or other drugs of misuse on either the eye or brain may result in an alteration of visual electrophysiology.

There is evidence from animal studies that methadone accumulates in the eye in the developing fetus. Pertschuk *et al* (1977) used an immunofluorescence technique to compare the eyes of adult rats exposed to methadone with neonatal rats that had been exposed to methadone *in utero*. 32% of the neonatal rats who had been exposed to methadone prenatally demonstrated positive immunofluorescence localised to the retina; by contrast no adult rats showed evidence of methadone staining in the eye (133). Similarly, Davis *et al* (1979) found that postnatal methadone administration to both rats and frogs led to an accumulation of methadone and its metabolites in the eye tissue at a concentration 100 times greater than that in the blood (134). Immunofluorescence studies have also demonstrated neuronal staining for methadone in the brain tissue of methadone addicts who had died of an overdose and in rats administered methadone (135,136). In both studies, methadone-staining was seen in the hypothalamus, thalamus, hippocampus, amygdale, cerebellum and brain stem.

Methadone exposure also leads to an alteration of neurotransmitters within the brain. Guo *et al* (1990) investigated the effect of prenatal methadone exposure

on acetylcholine levels in the brains of neonatal rat pups and found reduced acetylcholine content in the striatum (137). Robinson *et al* (1996) demonstrated increased acetylcholine turnover in rat pups exposed to methadone *in utero* compared to controls (138) and choline acetyltransferase expression (the synthesising enzyme for acetylcholine) was reduced in the brains of rat pups exposed to methadone *in utero* compared to controls (139). The combined or individual effects of reduced acetylcholine content, increased turnover and reduced choline acetyltransferase activity may lead to a depletion of acetylcholine in the brain with a resultant alteration in visual electrophysiology. In a follow-on study Wu *et al* (2001) found methadone exposed rat pups to have a 40-50% reduction in neurotrophic nerve growth factor compared to controls, suggesting a mechanism for the alteration in cholinergic neurons shown in other studies (140).

Methadone and morphine exposure have an adverse effect on opiate receptor binding in animal models (141,142): prenatal exposure to methadone resulted in reduced μ-opioid binding affinity in the neonatal rat pup (141) and prenatal morphine administration significantly altered regional development of opiate receptors in the brains of the rat pups (142). Perinatal morphine administration also causes a reduction in neuronal packing density in the somatosensory cortex and preoptic area of the hypothalamus with morphine-induced reduction of basilar dendritic growth in cortical pyramidal neurones (143).

Although no similar studies exist in human neonates exposed to methadone *in utero*, any combination of the mechanisms described above could lead to an alteration in visual electrophysiology in the human newborn infant.

6.3 Six month follow up

6.3.1 Growth parameters

There were no significant differences in weight or head circumference between drug-exposed and comparison infants when seen for assessment at six months of age, reflecting normal postnatal growth. Importantly, drug exposed infants had demonstrated catch up head growth. A previous study also documented catch up

weight and head growth by 18 months of age in children who had been exposed to opiates *in utero* (38): that study found that children did not demonstrate catch up in longitudinal growth; length was not recorded as part of the current study protocol.

6.3.2 VEPs

Six month follow-up demonstrated that VEP abnormalities persisted beyond the neonatal period, with drug-exposed infants having smaller amplitude pattern responses with delayed latencies. Drug-exposed infants also had significantly fewer VEP responses to the small check size in keeping with the clinical picture of reduced visual acuity and delayed visual maturation.

The fact that VEP abnormalities persisted to six months of age suggests that the effect is not due to residual circulating opiate and is consistent with a permanent teratogenic effect of prenatal drug exposure on the developing visual system. By six months of age no infants were on treatment for or had symptoms of NAS.

6.3.3 Visual outcome

A significant proportion (40%) of the drug-exposed cohort failed the clinical visual assessment at six months of age, even after correcting for excess alcohol exposure *in utero*. Infants exposed to methadone *in utero* were over five times more likely to have a clinical visual abnormality at six months of age than non-exposed infants (relative risk of 5.1). There was no association between visual outcome and either NAS or drug exposure group, suggesting that all infants born to mothers on the methadone programme during pregnancy are at risk of visual problems, regardless of maternal illicit drug use.

Nystagmus

The overall incidence of nystagmus in infants who had been exposed to methadone *in utero* was 11%. By comparison, Lloyd *et al* (2006) found an incidence of nystagmus of 5% in a population of opiate exposed infants (4). Infantile nystagmus is extremely rare in the general population: the

Leicestershire nystagmus survey found an incidence of 0.24% (24 cases per 10,000 population) (144). The incidence of nystagmus in infants who had been exposed to methadone *in utero* was therefore 50 fold that of the general population.

Pilot Study 4 found that significantly more infants who had a history of NAS demonstrated nystagmus compared to those without a history of NAS (92% versus 38%, p=0.017) (125); this finding was not replicated in the main study (p=0.482), possibly due to the smaller number of infants in the main study with nystagmus (n=9).

It has been proposed that infantile nystagmus may be caused by visual deprivation, resulting in oculomotor development outpacing sensory development (145). A developmental model of infantile nystagmus suggests that it develops as a response to reduced contrast sensitivity in an early "critical period". An alternative explanation may be abnormal μ-opioid receptor binding in the cerebellum of the developing brain (64). Animal studies and post-mortem human studies have demonstrated neuronal staining for methadone in the cerebellum (135,136). Administration of opioids to healthy human subjects leads to a temporary disruption of ocular fixation, resulting in nystagmus, possibly due to an effect on opioid receptors within the cerebellum (146).

Strabismus

Disruption of coordinated binocular vision early in life leads to strabismus and varying degrees of amblyopia, with the extent of impairment dependent on the time of onset, duration and type of visual deprivation (147). Twenty-five percent of the methadone-exposed cohort had strabismus, which is in close agreement with findings reported elsewhere (61,62). This is much higher than in a similarly-aged general population: screening of 38,000 infants showed a prevalence of strabismus of 1.3% (71). The proportion of children in the main study with strabismus who had NAS or not did not differ: 13/41 versus 7/40, Fisher's exact test, p=0.138, supporting the finding by Gill that strabismus does not appear to be related directly with NAS (61).

Delayed visual maturation

DVM was diagnosed in 14% of infants exposed to methadone *in utero*. DVM is generally a retrospective diagnosis made in infants who show delayed visual development for their postnatal age but then demonstrate catch up. Since the follow up assessment did not take place until six months of age, it is possible that some cases of DVM could have been missed. Both the clinical diagnosis of DVM and immature VEP morphology demonstrated in the neonatal period suggest that prenatal exposure to methadone and other drugs of misuse causes a delay in the development of normal visual processing during the fetal and/or early neonatal period.

Fundal findings

Only two children in the retrospective case series (Pilot study 4) demonstrated fundal abnormalities. Since dilating eye drops were not used in the six month follow-up visual assessment, only infants who were seen by colleagues in ophthalmology had dilated fundoscopy undertaken, of whom only one had an abnormal examination documented (hyperplastic optic nerves). This is very much in contrast to the ophthalmic manifestations of fetal alcohol syndrome, where over half of children demonstrate optic nerve hypoplasia.

Cerebral visual impairment

CVI was diagnosed in 25% of children in the retrospective case-series. CVI causes problems with processing of complex visual scenes and visually guided movement (72-74). This results in children having difficulties in picking out objects from a visually crowded scene, recognising faces and emotions, reading and copying, and difficulty with steps, curbs and floor boundaries. These difficulties can affect performance and behaviour and could be one mechanism for the reported developmental delay and behavioural problems recognised in these children. This was the first time that CVI had been reported in association with prenatal opiate exposure and is likely to be an underestimate as CVI is commonly diagnosed around school entry age (125). The cohort of infants in the main study was too young at the six month follow-up to assess for CVI, highlighting the importance of longer term follow up of this cohort.

Effects of different drugs of misuse

Mulvihill *et al* (2007) suggested that infants exposed to a combination of opiates and benzodiazepines may be at higher risk for visual abnormalities (64). They proposed that concurrent use of benzodiazepines may prevent the up regulation of μ-opioid receptors in the brain where opiates commonly bind. In this study 89% of infants who developed nystagmus were know to have been exposed to benzodiazepines in addition to opiates *in utero*, this was higher than the proportion of infants exposed to benzodiazepines *in utero* who did not develop nystagmus (89% *vs* 68%), but the difference was not significant (p=0.268).

6.3.4 Neurodevelopmental outcome

Infants exposed to methadone *in utero* demonstrated reduced neurodevelopment quotients compared to control infants matched for birth weight, gestation and socio-economic group, even after correcting for maternal smoking, antidepressant use and excess alcohol consumption during pregnancy. This was a global delay with significant differences between all developmental sub groups. Neurodevelopmental delay has been widely reported in the literature in association with maternal opiate misuse and may be secondary to many different factors.

Social circumstances: Neurodevelopmental outcome in these infants may be confounded by social circumstances. Factors associated with drug misuse such as smoking, alcohol misuse, poor nutrition, housing and education all have potential adverse effects on infant development. To try to correct for these factors the controls were matched to cases for socioeconomic group and regression models used to correct for the potential confounding effects of maternal smoking, maternal antidepressant use and excess alcohol use in pregnancy. We cannot however rule out a specific impact of parental addictive behaviour on the neurodevelopmental outcome of these children. By six months of age 76% of drug-exposed infants were in the care of their biological parents and 24% were accommodated (17% in foster care and 7% accommodated with a family member). No infant had been adopted. Interestingly, infants who were accommodated performed poorer on their neurodevelopmental scores than infants living with their biological parents: these infants were, however, more

likely to have been treated for NAS in the newborn period and to have been exposed to poly-drug misuse including benzodiazepines and stimulants *in utero*.

Visual problems: Visual impairment may cause delay of developmental milestones, particularly eye-hand co-ordination, and correction of visual abnormalities may lead to a subsequent improvement in development. Visual impairment was found to be an independent predictor of poor neurodevelopmental outcome and will therefore have contributed to reduced neurodevelopmental scores in these infants.

Direct effect of drug exposure: Post mortem studies in narcotic addicts who died from a methadone overdose show uptake of methadone in the hypothalamic nuclei, sensory-motor cortex, cerebellum, hippocampus and thalamic nuclei (135). There may therefore be a direct effect of prenatal exposure to methadone on the developing brain which contributes to the neurodevelopmental problems seen.

6.3.5 Proposed overall aetiology

The nature of the abnormalities described in this study (abnormal VEPs at birth and six months of age, reduced visual acuity, DVM, nystagmus, strabismus, and neurodevelopmental delay) suggests a detrimental effect of *in utero* drug exposure on the brain rather than an effect on the eye *per se*. Although ERGs were not recorded as part of the main study protocol, the majority of children in Pilot Study 4 had normal ERGs, suggesting that retinal function in these children is likely to be normal. A general toxic effect of methadone and other drugs of misuse on the brain could result in acetylcholine and neurotrophic nerve growth factor depletion and cause the VEP abnormalities demonstrated with associated poor vision from birth. Since good vision is required to develop binocularity and regulate gaze stabilising mechanisms, poor vision from birth could explain the clinical visual abnormalities described at follow up. This mechanism is well demonstrated in animal studies: newborn monkeys with normal vision develop nystagmus and strabismus after a period of forced blindness secondary to having their eye lids sutured closed for a period of time. Similarly in kitten models, binocular visual deprivation via surgical or optical strabismus resulted in a severe loss of cortical binocularity (148,149).

6.4 Study strengths

Pilot study and sample size calculation:

A major strength of this study was the undertaking of appropriate pilot work. A preliminary pilot study demonstrated the feasibility of measuring flash VEPs in newborn infants exposed to methadone *in utero* (122). This pilot work demonstrated the poly-drug exposure of these infants and provided data to inform the sample size calculation for the study. The pilot work undertaken to inform the follow-up phase of the study (Pilot study 4) ensured that the visual assessment protocol used was appropriate for the population under investigation (125). The large sample size of 100 infants for the main study allowed the assessment of individual drugs on the outcome measures and in particular allowed analysis of a subgroup of infants exposed to opiate alone.

Data collection and drug exposure:

Another strength of the study was the prospective nature of data collection and the comprehensive pattern of drug exposure obtained from maternal interview, maternal notes and toxicology samples. This is especially relevant for the follow-up phase of the study as the vast majority of studies investigating longer term outcomes in infants exposed to drug misuse *in utero* rely on retrospective collection of drug-exposure data.

Recruitment rate:

The high recruitment rate of 98% ensured that the study population was representative of the general drug-misusing population as a whole.

Control population:

To try to correct for potential confounders, the comparison infants were matched to the drug-exposed infants for birth weight, gestation and socio-economic group. To ensure that the comparison population was not exposed to excess alcohol *in utero*, meconium samples were collected for FAEE analysis.

Blinding:

Where possible, assessments were undertaken blinded to exposure status. As the chief study researcher it was not possible for the author to be blinded to the infant's exposure status but repeat VEP analysis (both flash and pattern VEPs) was undertaken by a second individual who was blinded to the infant's exposure status. Any discrepancy in opinion was referred to a third party who was also blinded. This should have ensured there was no bias in interpretation of the VEP results. Six month visual follow-up was undertaken in conjunction with an optician who was blinded to the infant's exposure status, thereby reducing bias for the visual follow up.

6.5 Study limitations

Confounders:

Children who have been exposed to methadone *in utero* are also commonly exposed to other adverse consequences associated with maternal drug misuse, such as maternal physical and mental ill health, poor nutritional status, smoking and alcohol consumption (1,6). Despite matching successfully for DEPCAT scores and using regression models to correct for maternal smoking and excess alcohol intake it is possible that there remain differences other than pre-natal drug exposure which could account for the differences seen between groups.

Blinding:

The Griffiths neurodevelopmental assessment was undertaken by the author alone and it was not possible for her to be blinded to the infant's exposure status as she was responsible for recruitment, data collection and follow-up organisation. It is therefore possible there could have been some bias in interpretation of the developmental assessment. To minimise this, a well validated developmental scale for the assessment was used.

Follow-up:

Retaining subjects from poor and disadvantaged backgrounds to follow-up studies is recognised to be challenging due to their chaotic lifestyles and frequent change of address. A 40% drop-out rate was predicted for the follow-up phase of the study to allow for this: actual drop-out rate was 21% in the cases and 48% in the controls, overall 30%. This compared favourably with other studies: Hunt *et al* (2008) had a drop out rate of 50% in their study of 133 opiate-exposed infants (38). Every effort was made to optimise study retention including telephone calls to parents, reminder letters sent in the post, reminder telephone calls regarding visits and taxi transportation to the study hospital. A high loss to follow-up may introduce bias as the results of those infants lost to follow-up may significantly alter the final results: in this case it is proposed that infants whose family life is so chaotic that they are uncontactable for follow up are unlikely to perform better than infants who attend for follow up.

Timing of follow-up:

Six months was chosen for the follow up visit as the majority of visual problems could be diagnosed by this age and it was early enough to allow intervention if required in children with problems such as significant refractive errors. The six month appointment also allowed completion of follow up within the two year time duration of the study. The retrospective case series identified a subgroup of children with CVI which is usually not diagnosed until childhood (72,73) and so the proportion of children with visual problems may be higher if these children were to be followed up for a longer period of time.

Six months of age is generally recognised as being too young to make an accurate assessment of neurodevelopmental outcome. Full assessment of neurodevelopment should ideally be made at approximately two years of age and in the pre-school year. It is possible that some children may develop neurodevelopmental problems not apparent at six months of age and in addition six months of age is too young to assess for behavioural problems which have been reported to be more common in drug-exposed children. It is therefore likely that the six month follow-up under represents the full scale of visual, developmental and behavioural problems affecting children of drug-misusing mothers.

As the study progressed it became apparent that it would be useful to follow-up this cohort of infants for longer term assessment and an Ethics Committee amendment was made to collect consent for future follow-up at the time of initial consent.

Drug-exposure status:

Maternal history is often unreliable as a method of detecting prenatal drug exposure. This study used a prospective five-point technique to determine drug exposure in the study infants (maternal case notes, maternal interview, maternal urine toxicology, infant urine toxicology, infant meconium toxicology). This provided a comprehensive assessment of drug exposure and compares favourably with other studies. There are however no toxicology samples which can be obtained from an infant which reflect first trimester drug exposure, and drug-misusing women often present late for obstetric care due to chaotic lifestyle and a reluctance to have involvement with social services. It is therefore possible that an infant could have been exposed to an illicit drug which was not detected, particularly in the first trimester of pregnancy.

6.6 Clinical recommendations

Infants who have been exposed to methadone *in utero* should be referred for a programme of visual assessment. Referral should be made from the neonatal unit to the local ophthalmology service.

Neonatologists and paediatricians should be made aware of the risks of visual problems in infants exposed to methadone *in utero* and should enquire about and screen for these opportunistically when seeing children in clinic. Dissemination of the results of this study has been undertaken via presentation of the findings at national meetings and publication in peer-reviewed journals. A Press Release was circulated secondary to one of the publications and this has generated more widespread media attention (Appendix 9).

Infants and children with proven ophthalmologic abnormalities should be referred for visual electrophysiology. There does not appear to be a role for VEP testing in the newborn period to help predict visual or developmental outcome.

All children who have been exposed to methadone *in utero* with developmental delay should be referred to ophthalmology, and assessment should include a structured questionnaire to assess for CVI.

6.7 Future research and controversies

The cohort of infants recruited for this study has prospectively collected data including neonatal and maternal demographics and drug exposure status and so would therefore be ideally suited to longer-term visual and developmental follow-up.

Longer term visual follow up should include assessment for CVI. Developmental assessment should also be undertaken to determine how many children with developmental delay have associated CVI. Future research should also address whether a developmental programme could improve outcome in children with developmental delay and CVI. Longer term follow up would also allow an assessment of how accommodation status impacts on the child's neurodevelopmental and behavioural outcomes.

Methadone is widely prescribed to pregnant opiate-dependent women. Use of methadone in pregnancy conveys various advantages to mother and infant including stabilisation of maternal lifestyle and reduced incidence of both preterm delivery and IUGR (5,10,11). However infants born to methadone-maintained mothers have a significant incidence of NAS and longer term adverse visual outcomes. Provision of optimal health care is always a balance of the risks and benefits of any therapy but we also have a duty to "first do no harm". Prescription of a substance that has been shown to have potential long-term teratogenic effects on the developing fetus must be critically reviewed.

Further study should therefore investigate alternatives to methadone treatment for pregnant opiate-dependent women. Acute detoxification may be unsafe in pregnancy and relapse rates are high. Buprenorphine is a synthetic opiate which is used worldwide for opiate dependence with theoretical advantages over methadone in the treatment of opiate addiction in pregnancy due to reduced placental transfer (150). Limited published data on the use of buprenorphine in pregnancy suggest a good safety profile, a trend towards a shorter hospital stay

and reduced incidence of NAS for the newborn infant as well as potential advantage in terms of infant neural development (121,151). Future studies should investigate the role of buprenorphine versus methadone on both short and longer term infant outcomes.

7 Chapter 7 Appendices

7.1 Appendix 1: Parent information sheet

Visual and brain function in infants born to drug-using mothers

Parental information sheet: Version 4 (Sept 2008)

You are being invited to allow your baby to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please read the following information carefully and discuss it with others if you wish. Ask us if anything is unclear or if you would like more information. Take time to decide whether or not you wish your baby to take part.

What is the purpose of the study?

Methadone is given to pregnant mums to help to stabilise their drug habit and improve their health in pregnancy. The benefit of taking methadone in pregnancy is that the baby is less likely to be born prematurely; the downside is that he/she may develop withdrawal symptoms after birth. At present we cannot tell which babies will get withdrawals, which is why we are planning this study. We believe that measuring vision and brain-wave activity (using tests which will not hurt the baby) will help to explain why some babies develop withdrawal symptoms, and may tell in advance which babies will be affected. This might help us to care better for babies in the future. Because drugs in pregnancy may affect babies' longer term vision and development, we would like to follow up your baby when he/she is 6 months old to check his/her vision and development.

Why has my baby been chosen?

We are asking all pregnant mums who are receiving methadone treatment and/or using other drugs in pregnancy and whose babies are born no more than four weeks early if their baby can take part in this study.

Does my baby need to take part?

No. It is entirely up to you. If you do decide that your baby can take part you will be given this information sheet to keep and be asked to sign a consent form. You will be free to withdraw from the study at any time and you won't have to give a reason. If you decide not to take part or to withdraw, this will not affect the standard of care that your baby will receive.

What will happen to my baby and myself if he/she takes part?

While your baby is less than four days old, we will record their VEP (visual evoked potential) and their EEG (electroencephalogram). Small pads with leads will be placed on their head using paste which is like Vaseline. More pads on their chest will measure their heart rate and breathing. We will record the EEG, which is brain wave activity, for about an hour and video your baby so we know when he/she is asleep or awake. We will then record more brain wave activity while showing your baby flashes of light (the VEP). The tests are completely painless. No needles are involved. It will take about 1½ hours and you will be welcome to stay with your baby.

From your baby: we will collect samples of urine and meconium (faeces) during the first few days after he/she is born. The baby's urine sample will be collected from a small bag stuck onto his/her bottom which is painless. The meconium will be collected directly from the nappy.

From you: we will collect a urine sample when you come into hospital in labour and a blood sample from the placenta (afterbirth) afterwards. We know that lots of mums take extra drugs in pregnancy besides the methadone; the results of these tests will be kept confidential and will not be given to anyone else: they will not affect the care that you receive.

These tests will help us to know which drugs are in your system and whether they reached your baby.

We will make an appointment to follow up your baby at 6 months of age to check their vision and development. This will usually be done at the Princess Royal Maternity but if you are unable to attend we will ask your permission to come and visit you. Follow up will involve a repeat VEP test and we will also check how well your baby sees. If there are any concerns about your baby's vision or development he/she will be referred to one of the specialists at Yorkhill Hospital.

What are the possible risks of taking part?

There are no risks to either yourself or your baby from taking part in this study.

What are the possible benefits of taking part in this study?

There is not expected to be any direct benefit to either yourself or your baby from taking part in this study. There is a possibility that we may identify a problem with your baby's vision or development which would not otherwise have been detected until later, in which case we will refer you to a specialist. Taking part in the study may help future babies if the results allow us to improve the way we look after pregnant drug using mums and their babies.

Suggestions and complaints

If taking part in this research project harms your baby, there are no special compensation arrangements. If your baby is harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain about any aspect of the way you have been treated during the course of this study, the normal National Health Service complaints mechanism may be available to you. You can put any complaint in writing to Mrs. Anne Snape, Patient Liaison Manager, North Glasgow University Hospitals Division, 84 Castle Street, Glasgow G4 0SF (telephone 0141 211 5112). If you have any suggestions to make regarding the study, please contact Dr. Helen Mactier (telephone 0141 211 5304).

Will my baby's taking part in this study be kept confidential?

Unless you have any objections, we will inform your GP that your baby has taken part in this study. Otherwise, all information collected about your baby during the course of this research will be kept strictly confidential.

What will happen to the results of the research study?

Information gathered from this study will be analysed and the results submitted for publication in a medical journal. Information may also be presented at scientific meetings. Your baby will not be identified in any presentation or written document.

Who is funding this research?

The equipment required to record the VEP has been purchased for the Princess Royal Maternity by the Glasgow Royal Infirmary Appeals Trust. None of the persons involved receives any money when a baby joins the study.

Who has reviewed this study?

This study has been approved by the Glasgow Royal Infirmary Ethics Committee.

Contact for further information

Dr Helen Mactier, Consultant Neonatal Paediatrician, can be contacted on 0141 211 5304 or via Glasgow Royal Infirmary switchboard (0141 211 4000). If you have any questions or concerns - please simply ask the midwife who is looking after your baby. Her name will be on a card on your baby's incubator.

Thank you for taking time to consider this research study.

Dr Helen Mactier and Dr Laura McGlone

7.2 Appendix 2: Letter to G.P.

Neonatal Unit,

Princess Royal Maternity,

8-16 Alexandra Parade, Glasgow.

Visual and brain function in infants born to drug-using mothers.

GP information Sheet Version 1, June 2008

Dear Dr.

Please be advised that your patient (name)……..

Date of birth……..

Hospital number……

Address…….

Is participating in the above study. This is a study of visual and cortical function in newborn infants exposed to methadone +/- other drugs in pregnancy. The infant underwent EEG and VEP recordings during the first week of life, and will be recalled for visual function testing at the age of 6 months.

If you have any queries, please contact Dr. Helen Mactier, Consultant Neonatologist at Princess Royal Maternity (telephone 0141 211 5249/5304) who will be happy to discuss them with you.

Yours faithfully,

Dr. Helen Mactier

7.3 Appendix 3: PRM neonatal abstinence syndrome guidelines

Introduction to the NAS policy

Neonatal Abstinence Syndrome (NAS) is a constellation of symptoms occurring in a baby as a result of withdrawal from physically addictive substances taken by the mother. These substances include methadone, benzodiazepines, opiates, cocaine and amphetamines as well as caffeine, nicotine and some antidepressant agents. The majority of infants with NAS in Glasgow will be withdrawing from opiates or opioids ± benzodiazepines. Almost all drug misusing mothers smoke in pregnancy; it is not known how much nicotine withdrawal contributes to symptoms.

Diagnosing NAS

Signs and symptoms of NAS include excessive irritability, in-coordinate sucking, vomiting, diarrhoea and poor weight gain. Rarely, convulsions may occur. The diagnosis of severity of NAS (and the need for pharmaceutical treatment) is largely subjective, but various scoring systems have been used in an attempt to standardise treatment. The scoring system currently used in Glasgow is the modified Lipsitz tool. The aim of treatment is to control symptoms to allow oral feeding, tolerable irritability and adequate weight gain. NAS is the likely diagnosis in an infant who demonstrates the signs and symptoms listed above and whose mother was known to have used addictive substances in pregnancy. Other common causes of excessive irritability can generally be excluded by careful history taking, clinical examination and measurement of blood sugar, calcium and magnesium.

Lipsitz Score Tool

Signs	0	1	2	3
Tremors (muscle activity of limbs)	Normal	Minimally increased when hungry or disturbed	Moderate or marked increase when undisturbed; subside when fed or held snugly	Marked increase or continuous even when undisturbed, progressing to seizure-like movements
Irritability (excessive crying)	None	Slightly increased	Moderate to severe when disturbed or hungry	Marked even when undisturbed
Reflexes	Normal	Increased	Markedly increased	
Stools	Normal	Explosive, but normal frequency	Explosive, more than 8 per day	
Muscle tone	Normal	Increased	Rigidity	
Skin abrasions	No	Redness of knees and elbows	Breaking of skin	
Respiratory rate / minute	< 55	55-75	76-95	
Repetitive sneezing	No	Yes		
Repetitive yawning	No	Yes		
Vomiting	No	Yes		
Fever	No	Yes		

Management of NAS

Simple measures to control symptoms of NAS include swaddling, the use of dummies and prolonged nursing. The pharmaceutical treatment of choice is the substance from which the infant is withdrawing.

Treatment should be started if the Lipsitz score $\geq$ 5 on two occasions 12 hours apart despite efforts to console the infant by nursing/carrying. Treatment may also be required if the symptoms are sufficient to cause poor feeding/ongoing weight loss after 5 days.

Pharmaceutical treatment:

This will depend upon the mother's drug use during pregnancy. Mothers will fall into 3 groups:

A. Opiate/opioid use only

B. Opiate/opioid plus benzodiazepine

C. Non-opiate/opioid drugs only

Groups A and B (Opiate/Opioid users)

Initial therapy - oral morphine solution 60micrograms/kg four hourly.

Escalating treatment - if symptoms are not controlled within 24 hours

- Increase oral morphine daily by 10micrograms/kg per dose to a maximum of 80micrograms/kg/dose.

- If symptoms are not controlled after 48 hours on the maximum dose of oral morphine add **phenobarbital** (dose as below).

Group C. (Non- Opiate/Opioid users)

Initial therapy - Start oral phenobarbital - loading dose 15mg/kg, followed by maintenance dose 8mg/kg once daily.

Weaning treatment: This should be commenced when the symptoms of NAS are adequately controlled. This may be defined as a Lipsitz score of $\leq$ 5 on at least one occasion in the past 24 hours. Also the symptoms may be considered controlled if the infant is able to be consoled if nursed and they are sleeping for periods of at least two hours between feeds. This latter approach is helpful if a baby is being weaned in the community without Lipsitz scoring.

Babies on Oral Morphine only

Each day, wean the oral morphine by 10micrograms/kg per dose. If symptoms worsen (Scores >5) during the weaning process, review the maternal drug history and consider addition of oral Phenobarbital rather than stopping or reversing the weaning of the morphine therapy. The aim is to reduce and stop the morphine therapy within the 1st 10 days of life as a delay beyond this time will necessitate a potentially avoidable admission to the SCBU.

Babies on Oral Morphine and Phenobarbital

Each day, if scores remain $\leq$ 5, wean the oral morphine by 10micrograms/kg per dose. Oral Morphine should be weaned completely before reducing the Phenobarbital therapy. Once the morphine has been discontinued the Phenobarbital may be weaned in hospital or, if the there are no other reasons for the baby to remain in hospital, as an out-patient.

7.4 Appendix 4: Standard Operating Procedure for recording VEPs

Check eligibility of infant:

> 36 weeks gestation

no congenital ocular abnormality

no signs of neonatal encephalopathy

infant clinically well

signed informed consent obtained

Infant care management:

Check infant's feeding schedule: VEP should be commenced shortly after completion of a feed. VEPs will be recorded in the consulting room in the Special Care Baby Unit (SCBU) in Princess Royal Maternity. Discuss planned recording session with SCBU staff and ensure that room is vacant. Affix "do not disturb" notice to consulting room door.

Invite parents to attend during the recording session. Avoid visiting period unless by discussion with mother (no visitors expected). Ensure that postnatal ward or SCBU midwifery staff (as applicable) are aware of the procedure.

Transport infant to SCBU consulting room in a cot. Take a bottle of infant formula unless mother is breast feeding. Ensure nappy clean and infant reasonably content. Offer additional feed if required.

Infants will be either placed supine in a cot or held by the parent or researcher during the recording.

Preparation of infant:

Examine electrodes for evidence of silver chloride coating and absence of visible scratches. Test electrodes by connecting together with conducting paste and performing impedance test.

Place scalp electrodes: The scalp electrodes will be placed relative to bony landmarks, in proportion to the size of the head, according to the international 10/20 system [1, 2] (Figure 1): The recording electrode will be positioned at a distance of 10% of the total nasion-inion distance above the inion in the midline (Oz position). The reference electrode will be positioned at a distance of 30% of the total nasion-inion distance above the nasion in the midline (Fz position). The ground electrode will be positioned on the mastoid bone below the ear.

Measure nasion-inion distance with a disposable measuring tape and note correct position for electrodes. Cleanse skin at electrode sites gently with a clean cotton bud and exfoliating paste. Apply conducting paste to electrodes and fix to the scalp with medical adhesive tape.

Scalp-electrode impedance will be measured prior to each recording and should be approximately equal with target levels of below 5 kOhms. If the impedance is greater than 10 kOhms, the electrodes will be repositioned and/or scalp gently recleaned. Overhead room lights should be switched off and the room illuminated by the wall-mounted angle poise lamp, turned towards the wall. The X-ray viewing box should be switched off.

VEP recording:

Switch on Espion® recording system and check that hand-held flash is plugged in and operating. Enter patient details including name, date of birth and hospital number.

The hand-held integrating sphere will be presented to the infant's eyes in the midline held against the infant's forehead. VIDI protocol Version 1.0 will be run which will deliver standard flash, bright flash and pulse wave and sine wave flicker. The protocol will be run twice to ensure reproducibility.

Documentation:

Awake/sleep state and degree of eye opening will be documented for each step of recording. Record relevant maternal and infant data on the case report form / database.

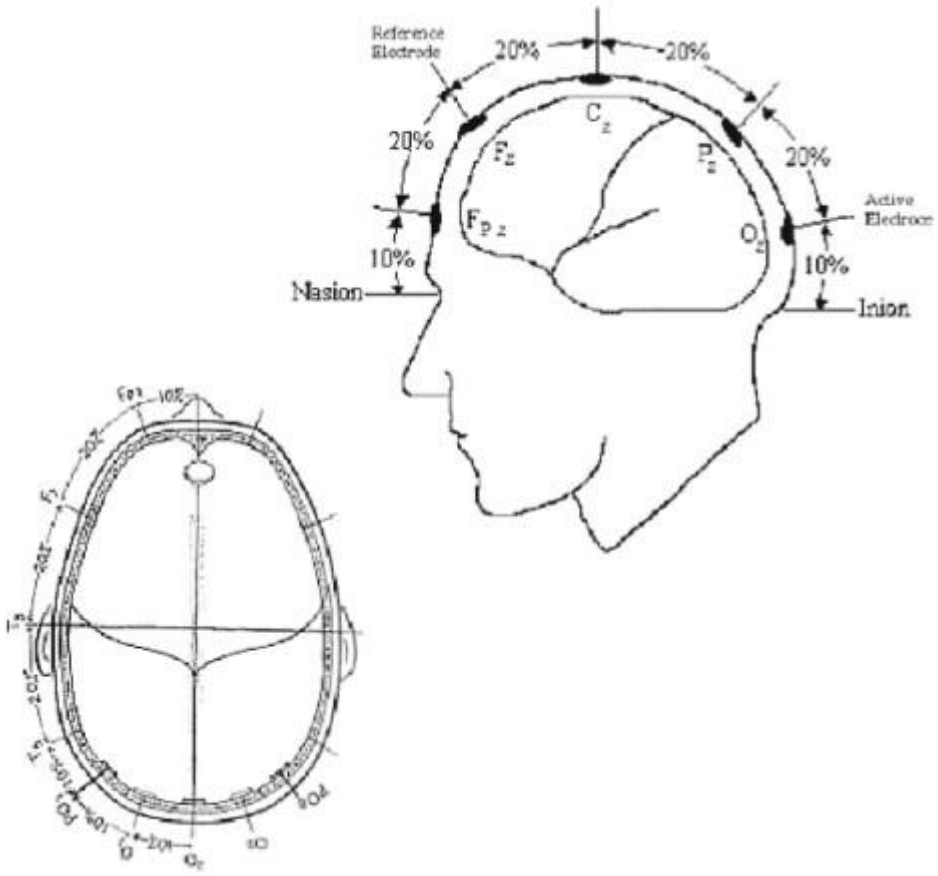
Completion of procedure:

Once recording has been completed the adhesive tape will be gently removed by the application of warm water and the electrodes removed from the scalp. Residual conducting paste will be removed with warm water. The recording session will be summarised with parents/carers. The infant will then be returned to the postnatal ward or SCBU.

Electrodes will be cleaned after each recording by soaking in cold water and gently rubbing off residual conducting paste. Electrodes will be sterilised after use by soaking in Milton® solution for 15 minutes. Electrodes will be soaked overnight in Milton® solution on a weekly basis to ensure re-chlorination.

VEPs will be stored in a password locked computer and data regularly backed up after each recording. The Espion recording system will be kept in a locked room within the PRM.

Figure 1:



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1. Harding G, Odom J, Spileers W, Spekreijse H. Standard for visual evoked potentials 1995. Vision Research. 1996; 36(21): 3567-72.

2. Odom J, Bach M, Barber C et al. Visual evoked potentials standard. Documenta Ophthalmologica. 2004; 108: 115-123.

7.5 Appendix 5: Letter to parent

Neonatal Unit,

Level 4,

Princess Royal Maternity,

8-16 Alexandra Parade,

Glasgow.

Date: …….

Dear

Thank you for agreeing to bring …….. for a vision and development check as discussed on the telephone.

We look forward to seeing you on

…….

Please come to the Neonatal Unit on Level 4 of the Princess Royal Maternity.

We will arrange a taxi to pick you up and take you home again as discussed on the telephone.

Kind regards

Dr Laura McGlone

Neonatal Specialist Registrar

Tel: 0141 211 5388

7.6 Appendix 6: Standard Operating Procedure for visual assessment

Parents will be invited to attend the Princess Royal Maternity for assessment. Assessments will be undertaken as close to six months (26 weeks) corrected gestational age as possible. Two professionals will be involved in assessment (research fellow and optician).

Tests will be carried out in a well lit room with the infant sitting on the parent/carers knee and will be timed to co-ordinate with the infant's feeding regime.

Eleven tests will be carried out in total subject to the child's co-operation. Each test has pass/fail criteria.

Symmetrical corneal reflexes: *

Lighted pen torch held 30cm from child's eyes. Attention gained on testers face. Corneal reflections should be symmetrical. Fail if constant asymmetry.

Pupil response:

Dim room. Cover each eye in turn and observe pupil constriction in response to a lighted pen torch. Allow 5 sec for response. Fail if no response.

Lateral tracking: *

Present a small toy in the centre of the field of vision 20-30 cm from the child's nose. Move toy at 10cm / second laterally to one side and note angle at which child stops tracking object. Repeat on other side. Can be repeated up to 3 times. Pass = 1/1 or 2/3 positive responses tracking to >20 degrees. Note type of eye movements and presence of NYSTAGMUS.

In addition, cover each eye in turn and observe for LATENT NYSTAGMUS.

Lateral field testing:

Tester kneels 60 cm in front of child so at same height as child. Use small high contrast toy on a stick. Move object from peripheral field inward, along an arc, towards the midline at a rate of 5 cm/sec and distance of 25cm from child's face. Note the angle from midline at which child looks to object. Repeat 2-3 times. Fail if complete absence of response either side.

Convergence to approaching object:

Present small toy 30cms from child's eyes. Bring toy towards nose at 2-5 cm/sec and watch for eyes converging. Fail if persistent lack of convergence.

Visual following of falling toy:

Attract attention to a large, colourful toy held by tester 60-90 cm away with outstretched arm. Observe whether child makes eye or head movement to ground as toy falls or immediately after toy has fallen. Can repeat up to 3 times. Pass is response on 1/1 or 2/3.

Batting/reaching:

A large colourful toy is held at arms length from child. Observe for attempts to bat / reach for toy. Fail is no attempt to obtain toy.

Acuity cards: *

Use Cardiff Acuity Test cards with pattern on one side and luminance matched grey field on other side. Present card 50cms from child's eyes. For each acuity level shuffle three cards then present card at child's eye level with the centre of the card at the tester's eye level. Observe infants' eye movements to estimate the position (top/bottom) of the pattern. If two correct estimates are made proceed to the next acuity level. The end point is taken as the highest level at which 2/3 cards are scored correctly and the equivalent Snellen acuity recorded.

Screening retinoscopy: *

Non-dilated retinoscopy is performed to screen for media opacities and gross refractive errors.

View retinoscopy reflexes in slightly dimmed room and note whether there are clear retinal reflexes in each eye (Fail = evidence of media opacity).

With the infant fixating the retinoscope, compare the speed of the reflexes between the eyes and between perpendicular meridians in each eye. Expected result is quick ‘with’ reflexes in all meridians showing a small lag of accommodation.

Engage the infant’s interest in a fixation target (toy on stick). Move the target in front of the retinoscope towards the child’s face and observe the reflexes using a retinoscopy working distance in the range of 50-67 cm. (Accommodation should neutralise and then reverse the reflexes ’against’ movement.). Pass =Neutral achieved with the target 10-20 cm in front of the retinoscope and equal between the eyes (no significant anisometropia) and between perpendicular meridians in each eye (no significant astigmatism).

Diffuse light reaction:

Sit child in darkened room. Shine a light source on the wall within child’s field of vision. Observe for head turn / eye movements towards light (5-10 sec for response). Repeat with light source on other side.

Defensive blink:

Tester sits facing child. Attract attention with wriggling fingers which are withdrawn until level with testers shoulder. Then move hand with fingers extended and palm forward rapidly (10cm/sec) towards child to 10cm from nose. Observe for rapid eyelid blink response. Can repeat up to 3 times. Pass is response on 1/1 or 2/3.

Tests highlighted with a ***** are to screen for the most commonly identified abnormalities detected in this population and should be prioritised if the infant has a limited attention span.

A fail in any test will result in prompt referral for formal ophthalmology assessment.

7.7 Appendix 7: Standard Operating Procedure for neurodevelopmental assessment.

Parents will be invited to attend the Princess Royal Maternity for assessment. Assessments will be undertaken as close to six months (26 weeks) corrected gestational age as possible. Two professionals will be involved in assessment (research fellow and optician).

Assessment will include:

Measurement of O.F.C.: Measured 3 times with a disposable tape measure, largest diameter recorded and plotted on appropriate growth chart.

Assess muscle tone and posture: Tone normal, hypotonic or hypertonic. Posture normal or abnormal.

Developmental assessment will be carried out using the Griffiths Mental Development Scales (Birth to 2 years) using the appropriate manual and recording sheets.

Locomotor development:

Examination prone (lifts chin, head, shoulders)

Examination supine (lifts head, shoulders, anticipates pull to sit)

Rolling (side to back, side to side, back to stomach)

Sitting with support (back firm, slight support, alone)

Crawling reaction (draws up knees, pivoting, tries to crawl, progress forwards or backwards)

Stepping reaction (dancing, one foot in front of other)

Playing with toes

Personal-social:

Smiles

Vocalises when talked to

Expresses emotions (2+)

Turns to person talking

Looks at mirror image

Spoon (holds, manipulates in play)

Awareness of strangers

Hearing/language:

Startles / listens to bell

Searches for sounds with head movements

Turns head deliberately to bell

Listens to tuning fork

Listens to conversations

Number of different sounds

Babble (two syllables)

Eye/hand:

Follows moving bell-ring (horizontally, vertically, in a circle)

Reaction to Ring (grasps, reaches for, grasps when dangling, secures when dangling, secures by string, dangles by string)

Looks for fallen object

Strikes objects together

Forefinger and thumb partially specialized

Performance:

Hand (to mouth, plays with fingers)

Reaction to Rod (holds, resists withdrawal)

Reaction to Cube (grasps, takes from table, holds two, manipulates, passes hand to hand, drops one cube for third)

Tissue paper (pulls, reaches for, plays with)

Lifts cup inverted over toy

A sub-quotient score will be calculated for each of the developmental sub-scales. A total general quotient score (G.Q.) will also be calculated using the Griffiths manual. Infants with abnormalities or developmental delay will be referred via the paediatric consultant to the appropriate hospital clinic (neonatal OPC, developmental clinic or neurology OPC). After completion of the Griffiths assessment all test material will be cleaned by wiping with sterilising alcohol wipes.

7.8 Appendix 8: Letter to ophthalmology

Neonatal Unit,

Princess Royal Maternity,

8-16 Alexandra Parade,

Glasgow.

Date: ……

Dear Jane,

The following baby was exposed to methadone *in utero* and was recruited to the VIDI study.

Name:

D.O.B.:

Address:

Tel. no:

Findings at the 6 month vision screening assessment were:

I would be grateful if you could arrange out-patient clinic follow-up for them.

Kind regards

Dr Laura McGlone

Neonatal Specialist Registrar

7.9 Appendix 9: Press release and media clip

BMJ Group

22 April 2010

BRITISH JOURNAL OF OPHTHALMOLOGY

Heroin substitute may be associated with wide-ranging sight problems

[Ophthalmic, clinical and visual electrophysiological findings in children born to mothers prescribed substitute methadone in pregnancy Online First Br J Ophthalmol 2010; doi 10.1136/bjo.2009.169284]

Children born to mothers prescribed the heroin substitute methadone during pregnancy may be at risk of wide-ranging sight problems, indicates a small study published ahead of print in the British Journal of Ophthalmology .

Babies of methadone mothers develop eye defects, say experts

Published Date: 28 February 2010

By Claire Smith

BABIES born to mothers who take methadone during pregnancy have developed a range of visual problems, according to a report by medical experts in Glasgow.

7.10 Appendix 10: Further reflections on methodology and future research

This was a cohort study as the exposure status was known definitively at the time of study recruitment. In the neonatal part of the study, infants who had already been exposed *in utero* to methadone were recruited and investigated for abnormalities of the VEP in the newborn period. These infants' demonstrated VEP abnormalities which were present at the time of study recruitment and a matched non-exposed group were recruited for comparison purposes.

In the follow-up study, the same cohort of methadone exposed and non-exposed infants were followed up prospectively to assess for clinical visual and developmental abnormalities. Therefore at the time of study recruitment the infants had a defined exposure status but were free of disease.

Validity of neonatal results:

The validity of the neonatal results can be considered under the headings of chance, bias and confounding. Chance was minimised by the large sample size recruited and the high significance level of the p-values obtained ($p<0.001$ for VEP amplitude and morphology).

Bias was minimised by the high recruitment rate of study participants, recruitment within a single hospital and matching of drug-exposed and comparison infants. In addition bias was further minimised as infants had an exposure status defined prior to study recruitment.

Confounding was minimised by the matching of drug-exposed and comparison infants. In addition, we explored for potential confounders and undertook regression analysis to correct for the effect of confounders on study outcomes.

Validity of follow-up results:

Bias was minimised in the follow-up study as exposure status was defined both prior to study recruitment and to occurrence of the disease, suggesting a temporal sequence between exposure and the disease. There was minimal error

in classification of exposure status (misclassification) as a result of the extensive toxicology undertaken in addition to history. The bias of non participation was minimised by the high recruitment rate of both drug-exposed and comparison infants. The bias of loss to follow-up is often a factor in prospective cohort studies and was minimised by the high retention rate of drug-exposed infants. Although there was a higher loss of comparison infants, there were no significant differences in demographic characteristics between comparison infants followed up and those not followed up, suggesting the groups were similar. In addition, published data suggest the incidence of visual abnormalities described in our comparison population to be representative of the larger population.

Confounding was minimised by matching of drug exposed and comparison infants and by restriction of study participants (to exclude the confounding effects of prematurity, congenital anomalies and illness). In addition, regression analysis allowed correction of other identified potential confounders.

Judgement of cause-effect relationship:

The above discussion highlights that it is unlikely that chance, bias and confounding are responsible for the statistical associations demonstrated in this study. We propose a cause-effect relationship between *in utero* methadone exposure and infant visual abnormalities. This is supported by the strength of association between methadone exposure and both abnormal VEPs and visual impairment: the relative risk of over five for both of these outcomes and attributable risk percent of over 80% makes it unlikely that another unidentified factor could account for the findings. The cause-effect relationship is also biologically credible: several animal studies have demonstrated a detrimental effect of prenatal methadone exposure on cerebral neurotransmitters and nerve growth factor which could have an adverse effect on early visual processing – it is entirely likely this effect also applies to human newborns. These findings are also consistent with other published studies in the literature, using alternative methodology in different geographic settings and populations', contributing to the growing body of evidence that prenatal methadone exposure is harmful to the developing fetus. In addition, the time sequence of the association supports a cause-effect relationship: *in utero* exposure definitively predated the onset of symptoms. Finally, there is evidence of a dose-response relationship as more

infants exposed to high dose methadone failed the clinical visual assessment than infants exposed to low dose; although this did not quite reach statistical significance. Assuming this causal effect of methadone exposure, over 80% of VEP abnormalities and clinical visual abnormalities could be attributed to methadone and therefore be eliminated if infants were not prenatally exposed.

Outline of future trial:

As our study has suggested a causal relationship between *in utero* methadone exposure and infant visual impairment, future studies should investigate alternatives to substitute methadone during pregnancy. Although 80% of the difference in outcome seems to be related to methadone exposure, it is possible some other unidentified confounders related to the lifestyle of drug misusing mothers could be partly responsible. This could be addressed by conducting a randomised, controlled trial programme to compare substitute buprenorphine treatment during pregnancy with substitute methadone. The primary outcome of this trial programme should be the incidence of clinical visual impairment and it should be powered to detect a reduction in the incidence of clinical visual abnormalities at six months of age in the buprenorphine-exposed group. A secondary outcome should be VEP abnormalities at six months of age. This trial programme would involve recruitment and randomisation in early pregnancy of opiate dependant women. Although the main outcome would be infant visual impairment, comparisons would also be made of pregnancy outcomes, neonatal outcomes (including birth weight, OFC, gestational age and development of NAS) and longer term developmental outcomes. Assessors should be blinded to the *in utero* drug exposure group. Such a study should ideally involve follow-up until school age to assess the natural history of visual and developmental abnormalities and assessment of CVI. A study of this nature would involve collaboration between obstetricians, neonatologists, ophthalmologists and developmental paediatricians. It would involve detailed data collection during pregnancy relating to smoking, alcohol use and additional illicit drug use and utilise urine and meconium toxicology collection to facilitate this.

8 References

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