INTERVENTION within the BRITISH AUTISM STUDY OF INFANT SIBLINGS (***i*-**BASIS**)**
PROTOCOL V1 18/07/2011

HYPOTHESES AND SPECIFIC AIMS

Aims

A pilot study to test the impact of a parent-mediated intervention for infants at high risk of autism. The study will gather information on:

a. Feasibility of recruitment and retention of subjects,
b. Feasibility of delivery of the intervention
c. Acceptability of the intervention for subjects and their adherence to the protocol;
d. Initial evidence of effect

Hypothesis

Can a developmentally targeted, environmental change (a structured psychosocial intervention) modify early behavioural markers of atypicality and targeted bio-markers related to brain function in a population of infants at-risk of autism spectrum disorder (ASD)?

BACKGROUND AND SIGNIFICANCE

Theoretical rationale

The "interactive specialisation" (IS) model [1] within cognitive neuroscience holds that that the "social brain" develops through an active process of postnatal interaction with the environment, (rather than through the innate linear unfolding of potential as proposed by a contrasting "maturational" hypothesis). The IS framework has successfully accounted for emerging evidence from studies of early face processing, oculomotor and attentional control, object processing, language, reading and developmental disorders [2]. IS postulates gradual changes in specialisation (the degree to which the responses of a cortical region are tuned to a certain class of input) and in localisation (the spatial extent of cortex activated following a stimulus presentation) in response to post natal activity. It predicts that:

1. Typically developing infants and toddlers are biased to orientate towards, attend to and learn from social stimuli. These biases contribute to the progressive post natal emergence of the typical cortical social brain network.
2. In autism spectrum disorders there is a failure or abnormality in one or more of the underlying mechanisms of biasing from early in life which then disrupts the typical emergence of the social brain network. This trajectory becomes further compounded by atypical interactions with the environment leading to the established pattern of symptoms observable by the age of diagnosis, and later social cognitive and communicative functioning.

This IS model is complimentary with evidence within developmental psychology and psychopathology on the importance of the early social environment (particularly the estimated 1000 hours of one to one social interaction in the first year with parents/caregivers) on later social and communicative functioning: and how these early environments can be disrupted in

atypical development. In neurotypical infants, the quality of parent child interaction has been shown to have importance for later non-social contingency tasks [3] and the development of joint attention, reciprocity, mutuality, for later socialisation and communication [4-6]. Evidence suggests that atypical neurodevelopment (for instance in Down syndrome, cerebral palsy, learning disability) is often associated with generally reduced parental sensitivity and increased intrusiveness [7, 8]: this may arise from difficulties in accurately interpreting infants' behaviours [9-11]. While a more directive parental style is not incompatible with sensitivity [12], supportive, contingent and sensitive parental responses appear to be central to the development of joint attention; and later language development is accelerated when the adult follows child-initiated and child-focussed topics for joint attention [12, 13]. This may be particularly important in children whose developmental impairments lead to difficulties in accommodating demands to shift focus and to regulate several competing demands on attention [14-16].The relevant parental skills can be trained [17].

With regard to infants at high risk of autism, two important possibilities arise from these convergent theories:

1. If we can identify the precise character of the interactional 'perturbations' related to early developmental atypicality in autism, this may lead to the elucidation of adverse interactional cycles with a negative effect on later development. (This would be an example of an 'evoked' environmental response within a heritable disorder).
2. Intervention to improve these interactional cycles could in theory therefore positively impact on later developmental trajectories. (This would not suggest that these cycles were a primary cause of autism, but that they might maintain or amplify a pre-existing vulnerability).

Our work and others' suggests that systematic experimental trials of an intervention in this context can serve an additional function of being a powerful method for illuminating basic developmental theory [18]. It has historically been difficult within developmental research to identify directions of causality within reciprocal interactions; in the model above, for instance, it is not necessarily easy to know which interactional consequences of atypicality are simply an epiphenomenon (with no bearing on future development) or which could themselves act back on the developing child with adverse consequences on development. An experimental randomised trial of intervention combined with a repeated measures observational design creates two parallel groups of infants for follow up, one of whom has had an targeted intervention changing a proposed developmental mediator. This is a powerful method for investigating causal processes in development [19].

Significance

As we outline below, our view is that both the basic neuroscience and the developmental psychopathology of autism are now at a stage where it is feasible and timely to initiate an integrated intervention/basic science study of this kind. Our proposed study represents a first step in this strategic line of research; which should allow the testing of competing predictions from the "interactive specialisation" and "maturational" hypotheses in specific aspects of autism development. The current study will provide tests of proof of concept and feasibility of the intervention and developmental measurement; plus initial systematic data to inform future larger scale studies in this area. It addresses the key issues relating to the early pathogenesis of autism and possibilities for strategic early prodromal intervention in the disorder.

PRELIMINARY DATA AND RATIONALE FOR THE STUDY

To rationally design and justify such an intervention study, we need to:

1. Identify candidate areas of early atypicality indexing developmental risk which should be targets for change during intervention. It is key to our design that these identified targets for intervention are simultaneously developmental processes or biomarkers that hold promise as being implicated in the developmental pathogenesis of the disorder.
2. Identify known interactional perturbations associated with early atypicality. These will then be the focus for intervention.
3. Identify best practice intervention strategies to impact positively on these interactions. Intervention in this context must be theory and evidence based and sufficiently intensive and targeted to produce the necessary environmental change.

We set out here the preliminary data and rationale in relation to each of these areas.

1. Candidate areas of atypicality: behaviour and neurophysiology

Our candidate areas for this study include 1) patterns of behavioural atypicality that may provide a prodrome for the behavioural phenotype of the broader autism phenotype; 2) relevant and evidence based 'biomarkers' which may be associated with the pathogenesis of autism. (We define these biological markers, or biomarkers, as observable properties of an organism that indicate variation in cellular or biochemical components, structure, or function and that can be measured in biologic systems or samples). Our review [20] highlights evidence for a subtle and variable expression of behavioural atypicality in the first year; and contrasts this with somewhat more consistent results when more fine grain measures are used ([21], and our results below). In this study we therefore investigate overt behavioural atypicality; but also combine this with measurement of more subtle expression that is detected using fine-grained behavioural measures and biomarkers.

a) Behavioural atypicality

Increasingly from the latter part of the first year and into the second, identified atypical behaviours in high risk autism sibs - in particular delayed emergence of early social communication behaviour such as joint attention, gaze monitoring and pretend play as well as abnormalities in attention - show association with later emergence of autism and the broader autism phenotype (BAP) [22-24]. By BAP we include aspects of the behavioural phenotype (and in adults also including face processing [25], theory of mind [26], executive function [27, 28], and central coherence [29]) associated with the autism phenotype and found not only in affected individuals, but also (to a lesser degree) in their genetic relatives. BAP is increasingly reported in infant siblings in children with autism who do not go on to a full diagnosis [20]. Evidence of these behavioural markers of atypicality can now be found in systematic measurements as young as six months of age and thus provide a one index of developmental risk and change in the population of infants at high risk. Our study addresses these behaviours in the latter part of the first year: this timing balances the reduced predictive value at < 1 year with the advantage of earlier intervention in relation to developmental plasticity.

b) Neurophysiological and saccadic movement biomarkers

Neurophysiological biomarkers are important because they may reveal subtle patterns of atypical brain function not evident in overt behaviour until later in development. Further, from studies of typical development it is evident that neurophysiological analyses such as ERP and EEG can reveal stimulus or task-dependent effects of brain processing in pre-verbal infants that are not evident using standard behavioural measures; and recent developments in eye tracking technology allow the detection of subtle differences in processing not revealed with other behavioural assessments. For this study we have selected neurophysiological, attention and eye tracking biomarkers based on (1) addressing domains of cognition and brain function relevant to autism, (2) compatibility with an existing large-scale study of infants at-risk for autism (our UK Infant Sibs Phase 1 study already undertaken) to enable greater power for some comparisons, and (3) demonstrated sensitivity (or likely sensitivity) to environmental manipulation. We will use

these biomarkers to assess specific hypotheses about the early developmental origins of autism, and its potential modulation by environmental manipulation.

We hypothesised earlier that in ASD there is a failure or atypicality in one or more of the underlying mechanisms of biasing toward social stimuli from early in life, which then disrupts the typical emergence of the social brain network. This trajectory becomes further compounded by atypical interactions with the environment leading to the established pattern of symptoms observable by the age of diagnosis. Based on the IS view, we further hypothesise that our intervention will prevent the compounding of early symptoms, without necessarily effecting the original atypical biasing to social stimuli in infants who go on to develop ASD. These hypotheses can be tested by assessing biomarkers of early social and non-social orienting and attention, and neurophysiological markers of cortical social stimulus processing. The modulation we predict is that only infants siblings assigned to intervention will go from atypical responses to stimuli at baseline to more age-typical responses at endpoint. Theory would suggest that there may be differential responses of these biomarker phenomena to environment intervention. Specifically it may be that the cortical social stimulus processing will be most responsive and non-social attentional control will be least sensitive to an intervention of the kind we will undertake. Identifications of such differential effects may be important for developmental theory and will also guide the more specific modeling of different intervention strategies to different deficits. There is already evidence that non-social attention and ERP measures of social processing can be influenced by experience in pre-school children. For example, Rueda et al [30] trained typically developing pre-school children in attention tasks and found later measurable changes in other attention measures and their ERP correlates, and de Haan et al [31] reported modulation of ERP face expression processing in infants reared by low affect mothers as compared to those reared by those with high affect scores.

Social attention and orienting. We will use a free-viewing task in which we simultaneously display five or six multiple objects including one face [32]. An eye tracking system registers the shifts in gaze of infants in response to these complex arrays. Previous work shows that orienting to faces dominates the attention of both at-risk and control groups of infants'. However, there are substantial individual differences in the degree of preferential orienting towards faces that are larger in the sib-ASD group. Follow-up of these infants at 24-months is currently underway and will be related to diagnostic outcomes.

Non-social attention and orienting is a variant of the "gap task". This task measures the "cost" of disengaging from a central stimulus in order to fixate a peripheral target. In a recent study with infants at-risk [33] reaction time did not differ during baseline trials for the two groups. However, the ASD-sibs group showed a longer reaction time to disengage during overlap trials than did controls.

Neurophysiological assessment of cortical social stimulus processing involves the scalp-recorded ERP and EEG correlates of processing a face compared to another complex stimulus, and a face with direct gaze compared to one with averted gaze, a known biomarker for young children with autism [34]. In previous work with infants at-risk for autism we have shown ERP effects over a posterior channel group [35]. In both infants at-risk and TD controls, the response to face with direct gaze was faster than to averted gaze for two early ERP components (P1 and N290), but infants at-risk showed significantly delayed response to direct gaze but not averted gaze in the later P400 response. Converging results were found using time-frequency analysis, where the control group showed earlier and more clearly differentiated induced gamma activity in response to the direct relative to the averted condition. Consistent with the ERP results, this analysis also indicates that the two groups differ mainly in their processing of the direct gaze condition. We predict that infants at-risk who receive intervention will be restored to the typical pattern of neurophysiological responses to faces observed in low-risk controls, whereas those that do not receive the environmental enhancement will continue to show an atypical pattern

characteristic of ASD. For all three biomarkers data from the proposed study will be directly compared to previously collected samples of infants at-risk and controls (N =50 for each; our UK infant sibs study phase 1) recorded with identical methods and at the same test ages..

2. Are there definable interactional consequences of atypical development in high risk autism siblings?

There are emerging detailed studies of early parent-child interaction in infant siblings at high risk of autism (A-sibs). One study of unstructured play examined the proportion of infant- and mother-led interactions at four months in 21 A-sibs and 21 typically developing (TD)-sibs [48]. During infant-led play, a quarter of A-sibs (and no TD-sibs) exhibited low synchrony, a finding which suggests that their mothers tended to show low affect matching. Three further studies using the structured 'still-face' paradigm (in which the mother interacts with the infant, then freezes on an expressionless face, then resumes interaction) at 4-6 months have also found atypicalities. Compared with TD-sibs, A-sibs showed more neutral affect at typically stressful points (during the still face - [48] or at reunion - [49]) and smiled less across all episodes [49] and a third of A-sibs showed diminished gaze to the mother's eyes relative to her mouth during the still-face episode compared with 1 in 24 TD-sibs [50] – although looking to the mouth predicted better language outcomes at 24 months.

Our own recent exploratory study [51] is the first to investigate the relationship between specific signs of behavioural atypicality in infancy and details of parent-child interaction. Using blind ratings of global mother-infant interaction, infant siblings of children with autism (6-10 months) with markers indicating potentially increased risk (top quartile scores on the Autism Observation Scale for Infants [22, 63]) were less attentive to or highly avoidant of their mother during interaction (3/11 or 27%) compared with low risk infants (21/44 or 48%). Mothers of these top quartile AOSI infants showed lower 'sensitive responding' (Fig 1: on a 7-point scale: mean 2.82; SD .98 v mean 3.80; SD 1.41; ANOVA: $F=4.70$; $p=0.04$) and low 'acceptance' (mean 2.64; SD 1.21 v mean 3.64; SD 1.57; $F=3.86$; $p=0.055$). These findings are consistent with findings from our investigations, at a later developmental age, of parent-child interaction in diagnosed pre-school autistic children. In one study, we used standardised observations of parent-child play in 27 children with core autism (mean age = 46 months, SD = 7.83) compared with data from a group of 24 typically-developing children (mean age = 23 months, SD = 6.50) group-matched on non-verbal ability. We found overall reduced scores on parental 'responsivity' in Autism compared with matched TD controls ([52], $p=<.05$; fig 2). In a separate study of 27 children with core autism, we found reduced parental communicative interactional synchrony with the autistic child, which was increased following parent-mediated treatment targeted at improving communication [53]. Independent studies in preschool age samples have shown that the degree of such parental synchronous response to the child's focus of attention and activity predicts joint attention and language outcomes in the child many years later [54].

Taken together these findings suggest that:

1. Relevant aspects of parent-child interaction can be measured during infancy
2. Global measures suggest specific interactional consequences to risk markers of even subtle atypicality in high risk infant sibs within the first year.
3. Such findings are consistent with interactional patterns in diagnosed autistic children
4. These interactional patterns can be modified with targeted intervention.

3. Modelling the intervention strategy

The above rationale implies the need for an intervention strategy that acts on the proximal social environment of the infant (in infancy, parent-child interaction) to best promote developmental trajectories. We will therefore target the intervention on key aspects of parent/child interactive behaviour that are: a) potentially relevant to development in autism and b) show evidence of

being amenable to change through intervention. While there is little evidence as yet as to the effect of a prodromal infancy intervention for high risk autism siblings, our prior work and our review of available evidence suggests candidate targets fulfilling these criteria in a number of areas:

a) Enhancement of parental synchronous responsiveness to infant signals. Our empirical research (section 2 above and [51]) suggests that global parental responsiveness and sensitivity to infant signals may be reduced in association with infant behavioural atypicality in high risk autism siblings. There is a strong evidence base regarding the effectiveness of targeted parent-mediated video-aided interventions in just this area to enhancing parental sensitivity and responsiveness, both in TD and generally 'at risk' infants. A metaanalysis of 81 studies (n=7636; [56]), including 51 RCTs (n=6282) of interventions to improve maternal sensitivity found that brief, focussed, personalised video aided intervention with parents was most effective (overall, d=.45). Greater effect was found with relatively briefer treatments focussed in the latter part of the first year (>6 months, d=.44; <6months d=.28). Substantive RCTs of such interventions on high social-risk non-autistic samples using focussed video feedback + education (3 home sessions at 6-9 months;[57] [58]) have found moderate to large effect sizes on improving maternal sensitivity and child functioning. These intervention strategies provide models of effective interventions in this domain at similar ages in infancy to our study population. They show that intervention can improve parent-child interaction in just the domain we have shown above to be implicated in high risk autism sibs. This evidence gives us the rationale for the core intervention approach in our current study. Furthermore, such a generic approach has the added value of being shown to be appropriate as a universal naturalistic intervention for parental enhancement independent of the risk status of the infant; maximising its ethical legitimacy and acceptability to parents.

However, while there is good evidence that such an approach will enhance generic parental function; the particular characteristics of development in infants at high risk for autism may well necessitate specific additional adaptations. We therefore plan supplementary components of the intervention (described next) which are more autism-specific, although they are of a character that do not either imply nor depend treated infants being prodromal for autism.

b) Parental synchronous response to infant communication. Convergent findings from studies of diagnosed autism in the pre-school period suggest the effectiveness of targeted communication based interventions in altering language communication and other aspects of social functioning. Our own studies in this area [53, 59] have contributed to this literature in the UK context. Members of the group are currently undertaking the largest intervention study yet undertaken into the effect of a targeted developmentally informed treatment on pre-school autistic disorder. Studies from our group have shown that a relatively brief parent-mediated video aided intervention can both improve autism symptoms [53] and child communication initiation through enhanced interactional synchrony and individually adapted communication between mother and child [60]. The logic of this evidence, based on enhanced analysis and adaptation of naturally occurring dyadic communication interaction, suggests that appropriate elements of our pre-school intervention may also be adapted into the infancy context. Such communication elements will supplement the generic 'sensitivity training' described above with autism-specific components known to be relevant in pre-school diagnosed autism; and we will be able to test them in the context of high risk sibling samples in infancy.

EXPERIMENTAL PLAN AND METHODS

Design.

A single blinded pilot RCT of two parallel groups: intervention and non-intervention. Research assessments made independently and blind to treatment status at baseline and following 5

months of intervention. Families will be recruited into the study through a recruitment database of the British Autism Study of Infant Siblings, a collaborative research network for the study of infants at-risk for autism (BASIS; REC reference 08/H0718/76). The design also builds on the ongoing BASIS Phase 1 study (REC reference 06/MRE02/73) where the previously approved experimental protocol will be used for the baseline and post-intervention assessments. The latter protocol has been run with over 100 families taking part in the study based at the Centre for Brain and Cognitive Development.

Recruitment and Consent

Siblings of autism probands will be sampled within the context of a collaborative research network, the British Autism Study of Infant Siblings (BASIS, see attached documentation), and would run in close collaboration with the MRC funded Phase 1 of the UK Baby Siblings programme at the Centre for Brain and Cognitive Development (CBCD), Birkbeck, University of London. Recruitment and ascertainment procedures to the BabyLab at CBCD are well established and ethically approved (see appendices). This gives a robust context within which to undertake an intervention trial in this innovative area for the UK, in terms of infrastructure support, ethics, acceptability to families and procedures. Additionally the trial benefits from incidental lab support (at no cost to the study) and the potential availability of a large body of data collected in the same laboratory on high risk infant siblings and typically developing infants for comparison and generalisation.

Selection criteria

Inclusion criteria: Families sequentially recruited to BASIS that live within therapist travel distance of treatment centres in London and Manchester (south east and north west UK; total population base of approx 14 million). We will not select by extent of phenotypic atypicality. Our rationale for this is: 1) current markers of atypicality are of lower predictive value before 1 year and do not preclude 'false negatives' with respect to autism development; 2) we consider that this increased uncertainty of prediction before the first year makes it ethically most appropriate not to select cases; selection by putative risk would necessitate that we feedback this fact to families, which would be ethically dubious. 3) preserving maximal dimensional variation in atypicality will allow us to examine the impact of intervention in all cases, even those who may not express clear behavioural signs (but may still be different at the level of biomarkers). This will give maximal power to our analysis. In sub-analysis we plan to investigate whether parental enhancement intervention would have better effect on behavioural atypicality as measured by AOSI: such a differential effect of environmental input would be an important finding.
Exclusion criteria: More than one sibling from the same family to preserve independence of subjects within analysis.

Feasibility

Within the 10 month recruitment period of the study, prior data suggests that 120 infant siblings of children with autism age 6-9 months will be recruited into BASIS. These families are recruited largely from self-referral following general advertisement in the media and UK autism networks. Systematic and anonymous feedback using questionnaires collected from families who took part in the CBCD baby siblings programme over the past 3 years has demonstrated that these families are already highly motivated to participate in autism research and recognise the potential broader impact of this infancy research. The target sample for i-BASIS is 50 and thus can be achieved with an opt-in as low as 40%.

Procedures

Randomisation: After research staff have confirmed eligibility and obtained consent, password-protected and encrypted details will be sent to the randomization centre (Christie Hospital Health

Care Trials Unit, Manchester). Allocation will be by minimization: controlling for treatment centre (Manchester/London); gender; age band at recruitment (6-7,5 months/7.5-9 months); baseline AOSI score (as variables with potential impact on treatment response or biomarker expression). The case will be assigned a study number and treatment allocation communicated separately to the treatment centre therapists.

Protection against other sources of bias. Researchers will be housed separately from staff involved in therapy and will attend separate meetings. Baseline assessment will be undertaken prior to parents being informed of treatment assignment. Research interviews will be constructed so as to avoid inadvertent divulging of information that could infer treatment status. The behavioural measure (AOSI) is rated by videotape blind to case details and treatment status. A random 20% of AOSI assessments (20 in total) will be check rated by an external blinded expert; one of the originators of the instrument. The assessment suite and materials used will be quite different in type and location to that used for the treatment intervention avoiding any familiarity effect for children in the treatment arm. Bias due to therapist effects will be minimised by frequent check on continuing therapist fidelity. All treatment sessions are videotaped. 5% of these sessions will be scrutinised by independent clinicians against fidelity criteria in the treatment.

Pre- and post intervention measures

Families taking part in the study will be invited to the babylab in central London. The common BASIS protocol of parent-report and standardised tasks will be administered (please refer to the BASIS research protocol for details). In addition, some experimental tasks will be also administered. These tasks are taken from a previously approved protocol of the "Longitudinal Study of Infant Siblings" (REC reference 06/MRE02/73). The tasks are detailed in the following section. Please refer to the previous research protocol for further details.

Intervention programme

The intervention design derives from a combination of developmental theory and the best current evidence for effective interventions for parent-child interaction in typically developing infants at high and low risk as well as in pre-schoolers with autism (see above). It is parent mediated and video assisted, integrating video-aided techniques to enhance parent-infant interaction [57, 61]. Core methods include: (1) A focus on dyadic, communicative aspects of the relationship, with a high degree of adaptation for each particular parent-infant dyad; (2) Videoclip viewing of 'successful' interactions, providing positive examples of sensitive, competent parenting; (3) Involvement of a trained therapist to frame observations to assist parent's self-reflection, and focus discussion and behavioural change. Parents' sense of efficacy is enhanced by active participation and the support given for their intuitive knowledge of their child. Intervention content focusses initially on enhancing parental observation, the attribution of communicative intent to infant behaviours that may be difficult to interpret and facilitating contingent parental responding and affective attunement [51]. To this foundation is added related components manualised and tested in our pre-school autism treatment studies [53]; promoting interactional synchrony and pre-linguistic skills such as joint attention. Our future findings in relation to specific interaction perturbations associated with atypicalities may lead to the inclusion of other specific elements.

The intervention will be manualised and delivered at the family home by specifically trained therapists with a psychiatry, psychology, speech and language therapy or therapeutic nursing/health visitor background. There will be twelve sessions at weekly intervals for three months and a further 4 sessions at two weekly intervals for two months; a total of 16 sessions. There is also 30 mins daily home practice for parent and infant between these sessions. In relation to intensity; less sessions are involved than in our current Pre-School Autism

Communication Trial at 2-5 yrs, because we bear in mind the age of subjects and evidence that overlong interventions in infancy may be counterproductive (56). On the other hand there are more sessions than in the TD infancy interventions reviewed above, bearing in mind the greater potential complexity of these high risk siblings in the context of autism.

Timing of assessments and intervention.
Recruitment will take place between 6-9 months. Intervention will start within 2-4 weeks of baseline. Follow up assessments will be completed within 1 month of treatment ending. Current evidence suggests that risk markers for development of autism become progressively clearer after 12 months. Our intervention is thus targeted at the cusp time at which more predictive risk atypicalities are thought to appear. This timing immediately prior to that therefore in theory maximises the opportunity for plasticity of development and preventative action whilst maximising the likelihood of significant atypicality to work on in the intervention. Furthermore, the timing of assessments coincides with the wider BASIS assessment protocol, resulting in the availability of extensive data on our putative neurophysiological biomarkers and their interactional consequences for comparison and reference and generalisation at the assessment points (see appendix). This timing therefore maximises the reliability of pre- post- measurement of neurophysiological biomarkers and their interactional consequences.

Measures (also see enclosed Summary of Measures document)
A. Generic BASIS measures. (Baseline and follow up (BASIS; REC reference 08/H0718/76).)
In addition to the specific measures related to study hypotheses (as below), participants will receive the core battery of measures common to the BASIS cohort (see appendix 1 for details). These include reference measures of (a) General cognitive and adaptive functioning: and (b) Social-communicative development which will allow group comparison with a larger representative sample. The Development and Wellbeing Assessment (DAWBA) [62] will be used to confirm the diagnosis of the older sibling according to BASIS protocol.

B. Acceptability and impact of intervention.
We will conduct detailed interviews with participating parents before and after the intervention, to gather information on their experience of the treatment and its acceptability. These will include:

1. Pre-Intervention
    i. Parental expectations of the intervention
    ii. General prior parental attitudes towards an intervention of this nature
    iii. Adequacy of information provided prior to delivery of the intervention

2. Post-Intervention
    i. Suitability of the environment in which the intervention was delivered
    ii. Responsivity of and rapport with the therapist
    iii. Acceptability of the sessions with respect to duration and frequency
    iv. Acceptability of the home practice expected between the sessions
    v. Evaluation of whether expectations were met exceeded or unfulfilled, with explanations of why this was the case.
    vi. Components of the intervention that were most useful, with explanations of why this was the case
    vii. Components of the intervention that were least useful, with explanations of why this was the case

3. Wider Impact of Intervention
    i. Consequences of the intervention, both positive and negative, for the relationship between the parent and the infant
    ii. Consequences of the intervention, both positive and negative, for the relationship between the parent and their child with ASD
    iii. Consequences of the intervention, both positive and negative, for the relationship between the primary caregivers

C. Behavioural atypicality (BASIS; REC reference 08/H0718/76)**..** The Autism Observation Scale for Infants (AOSI [22, 63]) is a battery of risk markers, focusing on precursors of abilities present in later the autism phenotype; including response to name, eye contact, social reciprocity, and imitation. Additionally the battery examines hypothesised precursor skills such as visual tracking and disengagement of visual attention. Initial data on the instrument at 12 months [22] showed prediction to autism diagnosis at 24 months with a sensitivity of 84% and specificity of 98%, using a 7+ marker threshold. Predictive markers included atypicalities in eye contact, orienting to name, social smiling, and prolonged latency to visual disengagement. Inter-rater reliability both for total scores and number of endorsed items is good to excellent at 6, 12 and 18 months; test-retest reliability is acceptable 12 months. AOSI will be measured at baseline and follow up in a lab setting with trained administrators. Training and reliability support will be undertaken by one of the originators of the instrument [22].

D. Neurophysiological and saccadic movement biomarkers (baseline and endpoint, (Longitudinal Study of Infant Siblings; REC reference 06/MRE02/73).
Measures will be the same at both timepoints with slight variations in the stimuli to ensure some degree of novelty. All of these tasks have been administered at both ages previously in our lab and continue to be age appropriate.
Social orienting and attention. We will use *eye tracking* to assess several aspects of social orienting, perception and cognition: *Face pop-out*: In this paradigm infants are presented with a series of visual arrays composed of five objects in different spatial locations on a screen. The objects include colourful cars, watches etc, and one photographic image of a face of the same size. Infants watch 12 different arrays each presented for up to 20 seconds. Data on 6-month old infants [32] showed that by several measures (time spent on each object, direction of first look, number of saccades directed to each object) typically developing infants spend proportionately more time on, or repeatedly returning to, the face stimulus. Again, data from the present study can be compared to larger samples of infants at-risk and controls obtained at the same ages with the same procedures. Saccadic responses are analysed based on eye-tracking by means of infra-red detection (Tobii-1750). All visual stimuli used will be designed to be well above acuity and contrast sensitivity thresholds for the age groups concerned. Key measures derived from these and tested pre- and post-intervention are: frequency of saccades and fixation duration (ms) towards a given stimulus, and more specifically to the face relative to competing stimuli averaged across trials.
Non-social attention: *The Gap task* has been studied in detail in infants, children and adults with and without ASD [2, 14] It involves measuring saccadic reaction time to a peripheral visual target, either in the continuing presence of a central fixation stimulus (overlap trials) or with a time gap between the offset of the central fixation stimulus and the onset of the peripheral target. Zwaigenbaum et al. [22] presented preliminary evidence that difficulty in disengaging from a centrally presented visual stimulus could be an early marker for later emerging ASD symptoms, a finding further supported by our own work showing group differences between ASD-sibs and controls at 9 months of age (Elsabbagh et al. submitted, IMFAR 2008). Our procedure uses improved methods from those previously employed, allowing us to also measure baseline and

cue facilitation effects. Key measures derived are the reaction time difference between conditions corresponding to disengagement (Baseline vs. Overlap) and facilitation (Baseline vs. Gap) effects.

Electrophysiological measures of cortical social processing:

The ERP paradigm efficiently combines a number of tasks we have previously developed for testing typically developing infants. EEG is recorded as infants view static photographs of female faces displaying direct or averted gaze and analysed using: Event Related Potentials (ERP) and Time Frequency Analysis of oscillatory brain activity in the gamma band (20-60 Hz). This paradigm compares faces to structured visual noise [64], and gaze shifts moving toward or away from the infant. Thus, this paradigm builds closely on our previous results from young children with autism [34] and typical infants and adults (see [65] for review) but also includes comparisons that will allow us to assess preliminary reports of atypical face and gaze processing in ASD-sibs as a group. ERP procedures and reporting follow established guidelines [66], with a few minor modifications specific to testing infants [2]. We have published direct comparisons between data recorded by the EGI net, and those recorded from conventional low-density systems [2]. With regard to the analysis of ERP data, we will continue to utilise two approaches. The first approach is conventional ERP data analysis in which the main step is finding the temporal coherence of the recorded EEG by averaging time-locked to relevant events (stimulus presentation or action onset). In the resulting ERPs, peaks and deflections can be identified in groups of neighbouring channels, and differences between experimental conditions in the latency or amplitude of these "components" are statistically evaluated. It is important to note that the presence or absence of differences between experimental conditions can be identified even if there are differences in the overall waveform between age groups or clinical populations.

E. Parent Child Interaction (BASIS; REC reference 08/H0718/76)***.***

A standard five-minute unstructured play paradigm will be employed, in which the mother is instructed to play with their infant as they would normally (on a floor mat, with or without toys). The videotaped interactions will be coded independently blind to risk status using a 7-point global rating scale, which has been modified from validated measures to suit our study group by combining the brevity/age appropriateness of the Global Rating Scales of Mother-Infant Interaction (GRS; [4]) and the autism-specificity and format of the 'Coding of Attachment-Related Parenting -for use with children with Autism' (CARP; [67]). The new scale, which has demonstrated feasibility and inter-rater reliability [51] comprises 3 maternal items (sensitive responsiveness, acceptance, appropriate affect), 3 infant items (attentiveness /avoidance, appropriate affect, positive vocalisation), and 2 dyadic items (synchrony/mutuality, quality of engagement). This will be measured using the standard 5 minutes free play paradigm between parent and infant video taped for blind coding. Coding will be undertaken on generic, language-specific, and atypicality-specific dimensions using adaptations of the CARP and GRS measures.

DATA ANALYSIS

General Issues:

Intervention and Developmental Analysis: The general approach to analysis will span the more pre-specified formula of RCT reporting of the intervention effect, for which the study has been powered, and the more exploratory developmental analysis within which we will be viewing the intervention as a randomised experimental manipulation of the social environment. In light of the sensitivity of measures to maturation all analyses will covary for age.

Data reduction: In general, standard scoring schemes and cut-offs will be used wherever possible. Systematic data cleaning and checking will be undertaken. Pro-rating of occasional missing items will be used in the calculation of item total scores.

Missing Data: Analyses of data involving missing measures will be undertaken wherever possible either by maximum-likelihood or by the use of multiple imputation, carried out using the

iterative chained equation approach as implemented in Stata [68]. Both approaches allow for potentially selective but non-informative attrition.

Effect Estimators: Analysis of the intervention effect element of the study will initially use an intention to treat (ITT) analysis to compare endpoint group effects using apriori hypotheses related to each measurement domain. Where non-compliance is a concern or parent training proves to be of variable success, alternative effect estimates will be calculated, such as the Local Average Treatment Effect [18]. These continue to exploit the randomised treatment allocation undertaken, and are also valuable in obtaining estimates of mediation unbiased by residual confounding.

Significance and Precision: In the light of the modest sample size and concern about the appropriateness of asymptotic estimators of precision and significance, bootstrap methods will be used.

Analysis of Behavioral Measures

The behavioural measures analysis will focus in the first instance on the simple total markers derived from the AOSI. We will make use of the structural equation modelling set up that is equivalent to Analysis of Covariance. This allows the addition of age at assessment as an additional covariate to both baseline and endpoint scores.

Hypothesis: the intervention will reduce the total specific precursor markers for ASD.

Towards the end of the study information will have accumulated follow up data to enable more confident assessment of the degree of any ASD symptomatology. We will then use signal detection methods to estimate and refine the performance of early biological and cognitive markers against available current best estimates of ASD as data is acquired. In view of the modest sample size leave-one-out cross-validation methods will be used. Given current estimate of recurrence rates, the sample size of this study will not have the power to test definitively the effect of infancy intervention on eventual diagnostic autism outcome. However a positive result from this study would support larger sample studies over a longer time period to do this.

In a number of areas of skill, development is characterised by the timed achievement of milestones. Available data is often interval censored, indicating a window of time in which a milestone was achieved, for example from repeated questionnaires on current status (as in the Vineland) or imperfect recall (as in the ADI-R). We will use multivariate interval censored survival methods to assess the effects of the intervention in hastening skill acquisition/normative development or delaying onset of ASD characteristic symptoms.

Parent-child interaction measurement is our hypothesised mediator of the treatment effect. Dimensional summary measures from the coding of interaction with the sibs will be analysed using MANOVA and Structural Equation Models to test and examine the structure of the association among these variables at baseline, the structure of the pattern of association in their change over time, the difference according to risk group, and the differences associated with the intervention.

Saccadic movement and Neurophysiological analysis. The logic of this overall design is that we will be able to compare (1) pre- and post intervention measures, (2) developmental changes in the no intervention group, and (3) the two groups at the endpoint. In addition to comparisons with our substantial existing data set on these same tasks (UK infant sibs phase 1, which will include FU to diagnosis during the course of this study), we will thus be able to describe the typical developmental trajectory in these measures, the at-risk infant (broader phenotype) trajectory, and the effects of our environmental enhancement on the at-risk infant trajectory.

Analyses will test for group by condition interactions, controlling for age through parametric analysis using general linear models.

Hypotheses:
Face pop-out: Levels of pre-intervention fixation duration towards the face will increase relative to distactors in the experimental group but not in the control group.
Gap task: controlling for the effects of age, there will be shorter latencies to disengage in the overlap condition post-intervention relative to pre-intervention in the experimental group relative to the control group, but there would be no change in the baseline condition. The disengagement effect post-intervention would be smaller in the intervention group relative to the control group.
ERP task: controlling for the effects of age there will be shorter latencies in the P400 component in response to direct gaze in the experimental group relative to the control group. On the other hand, there would be no change in the response to the averted gaze condition.

In secondary exploratory analysis we will also be able to test general task parameters (e.g., response in early visual ERP components and overall levels of orienting in saccadic reaction time tasks) to examine whether more general effects can also be observed in the experimental relative to the control group. Additional analyses will examine the extent to which neurophysiological measures and biomarkers are associated with each other and with change in brain function.

Sample size and power
Behavioural markers. The proposed pilot two-group (n=25 per group) intervention study would have a nominal power of 80% (two-tailed alpha=0.05) for an outcome group difference of 0.8SD on the behavioural phenotype measure (AOSI); slightly smaller than that we have found in our intervention for diagnosed children [53].
Saccadic movement and Neurophysiological analysis. Gap task Disengagement effect: GLM with condition as within (Baseline, Overlap) and Group as between (Sib-ASD vs. Control). N=15 sib-ASD and 16 ctl yields effect size (partial eta squared) = 0.17 and observed power of 0.66 (at alpha=0.05). Gaze ERP latency of P400 component: GLM with condition as within (Direct, Averted) and Group as between (Sib-ASD vs. Control). N=17 sib-ASD and 19 ctl yields effect size (partial eta squared)=0.16 and observed power of 0.70 (at alpha=0.05).
We can be therefore confident that the N of 25 in each group in this study will substantially exceed this in power.

Timeline

Month	Activity	Personnel	Milestones/output
1-4	Start up	Research assessors (RA) Research Treatment Therapists (RTT)	Training of RAs in assessment methods Training of RTTs
4-24	Recruitment, consenting	RAs	Recruitment of minimum N=50 subjects into randomised trial by Month 24 (= 2.5 subjects/month)
5-26	Baseline assessments	RAs	
7-29	Treatment intervention	RTTs	Each treatment cycle @ 5 months. Total of n=25 treatments minimum

			over 23 months
10-30	Follow up assessment	RAs	
7-30	Ongoing coding/processing of neurophysiological data	RA (Birkbeck), PIs (MJ,ME)	
7-30	Ongoing coding of parent child interaction data	RA (Manchester), PIs (MWW, JG, VS)	
31-34	Data analysis	RA (Statistics), PI (AP)	
33-36	Report preparation	PIs	

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