BMJ open
accessible medical research

UP-BEAT (Upper Limb Baby Early Action–observation Training): protocol of two parallel randomised controlled trials of action–observation training for typically developing infants and infants with asymmetric brain lesions

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ABSTRACT

Introduction: Infants with asymmetric brain lesions are at high risk of developing congenital hemiplegia. Action–observation training (AOT) has been shown to effectively improve upper limb motor function in adults with chronic stroke. AOT is based on action observation, whereby new motor skills can be learnt by observing motor actions. This process is facilitated by the Mirror Neuron System, which matches observed and performed motor actions. This study aims to determine the efficacy of AOT in: (1) influencing the early development of reaching and grasping of typically developing infants and (2) improving the upper limb activity of infants with asymmetric brain lesions.

Methods and analysis: This study design comprises two parallel randomised sham-controlled trials (RCTs) in: (1) typically developing infants (cohort I) and (2) infants with asymmetric brain lesions (eg, arterial stroke, venous infarction, intraventricular haemorrhage or periventricular leukomalacia; cohort II). Cohort II will be identified through a neonatal ultrasound or neonatal MRI. A sham control will be used for both RCTs, taking into consideration that it would be unethical to give no intervention to an at-risk population. Based on a two-tailed t test of two independent means, with a significance (α) level of 0.05, 80% power, predicted effect size of 0.8 and a 90% retention rate, we require 20 participants in each group (total sample of 40) for cohort I. The sample size for cohort II was based on the assumption that the effect size of the proposed training would be similar to that found by Heathcock et al in preterm born infants (n=26) with a mean effect size of 2.4. Given the high effect size, the calculation returned a sample of only four participants per group, on a two-tailed t test, with a significance (α) level of 0.05 and 80% power. As cohort II will consist of two subgroups of lesion type (ie, arterial stroke and venous infarction), we have quadrupled the sample to include 16 participants in each group (total sample of 32). Infants will be randomised to receive either AOT or standard Toy Observation Training (TOT). Both interventions will be of 4 weeks' duration, from the infant's 9th–13th post-term week of age. Three sessions of 5 min each will be performed each day for 6 days/week (total of 6 h over 28 days). Parents of the AOT group will repeatedly show the infant a grasping action on a set of three toys, presented in random order. Parents of the TOT group will show the infant the same set of three toys, in random order, without demonstrating the grasping action. At 14, 16 and 18 weeks, the quantity and quality of reaching and grasping will be measured using the Grasping and Reaching Assessment of Brisbane; symmetry of reaching and grasping will be measured using the Hand Assessment of Infants (HAI) and pressure of grasping for each hand with a customised pressure sensor. At 6 months' corrected age, the primary outcome measures will be the HAI and Bayley Scales of Infant and Toddler Development (third edition; BSID III), to measure cognitive and motor development. At 8 months, HAI and EEG will be used to measure brain activity and cortical coherence. At 12 months, the primary outcome measures will again be HAI and BSID III.

Dissemination: This paper outlines the theoretical basis, study hypotheses and outcome measures for two parallel RCTs comparing the novel intervention Action–observation training with standard TOT in: (1) influencing the early development of reaching and grasping of typically developing infants and (2) improving the upper limb motor activity of infants with asymmetric brain lesions.

Trial Registration: ACTRN1261100991910. Web address of trial http://www.ANZCTR.org.au/ACTRN12611000991910.aspx

BACKGROUND

Infants with asymmetric brain lesions (eg, intraventricular haemorrhages, periventricular leukomalacia, arterial strokes and venous infarctions occurring on one side or more involved on one side of the brain) are at high risk of developing congenital hemiplegia by the end of their first year of life. The incidence of asymmetric brain lesions at birth is 1–2 of every 1000 newborns.[1]

Congenital hemiplegia is the most common type of cerebral palsy (CP), with a prevalence of 1 in 1300 live-births.[1] The economic impact of CP is substantial. In 2007, the financial cost of CP was estimated at AUS$1.47 billion, with $124.1 million of that cost directly attributed to intervention costs.[2] Approximately 43% of these costs are covered by the families of individuals with CP, with the remaining 57% being covered by various levels of government.[2]

The main focus of early intervention for infants with asymmetric brain lesions who may progress to classification of unilateral CP is very early and accurate detection of the brain lesion, followed by provision of an enriched environment and training to maximise upper limb function during critical periods of development. The challenge for clinicians and researchers is the limited number of tools available to identify the problem and measure progress, as well as a paucity of evidence for efficacy of very early upper limb rehabilitation.

Broadly speaking, there are two common clinical presentations of asymmetric brain lesions, early or delayed. Early presentation consists of a perinatal onset of neurological symptoms, or seizures, or reduced movement at 24–48 h postbirth with verification on cranial ultrasound and/or MRI of the presence of an asymmetric brain lesion. Specific imaging protocols may be needed for diagnosis in the early phases, such as diffusion MRI to identify an acute stroke in the first hours or days.[3] In a delayed presentation, the infant may have an initially uncomplicated perinatal course and may not show signs of stroke or asymmetric brain injury (aBI) until 3–7 months of age, when unilateral weakness and early hand preference start to manifest.[4 5]

Currently, the most predictive tools for early diagnosis of CP are a combination of brain MRI at term and Prechtl's Assessment of General Movements (GMs) in the fidgety period (at 12 weeks post-term).[6] Specifically, GMs at 1 and 3 months post-term age are highly associated with white matter abnormalities on MRI at term age.[7] GMs are a well validated and reliable tool that is more sensitive at predicting CP than other motor assessments used in infancy.[8 9] They are also useful for prediction of minor motor difficulties.[10] Neuromotor assessments (such as the GMs) utilised in the neonatal period (<4 months post-term) have strong validity to detect CP in infants born preterm, when correlated with criterion assessments at 12 months; corrected age (CA; such as the Bayley Scales of Infant and Toddler Development (BSID III); 8). Although abnormalities in GMs are likely to be evident during the early writhing period (<6–9 weeks post-term) and the fidgety period (9–20 weeks post-term), asymmetries are only visible during the fidgety period.[11–13] Asymmetries in fidgety GMs around 12 weeks post-term can be the first definitive clinical sign of hemiplegia.[11–13]

Very early detection of hemiparesis frequently requires serial evaluation of subtle signs of interlimb differences or asymmetries in upper limb reaching (both spontaneous and purposeful) and grasp strength.[14] Both bimanual and unimanual reaching with early strong hand preference at 4–6 months of age can be considered to be a strong sign of early hemiplegia.[4] Studies of infants who have sustained an early perinatal stroke before 4–7 months CA have suggested that until reach to grasp behaviours have emerged, an asymmetry may not be clearly evident and hemiparesis not confirmed.[15 16]

Early intervention for infants at risk of developing congenital hemiplegia is considered to be very important; however, standard rehabilitation programmes generally start after 6 months of age due to delayed detection. A further consideration regarding the timing of start of intervention is that important phases of brain reorganisation may have already occurred.[17 18]

Current approaches to rehabilitation in congenital hemiplegia in infants focus on toy presentation and sensory stimulation of the limb to encourage spontaneous reaching and grasping; however, the challenge is to obtain active movement from the impaired limb. A new approach utilising action observation to stimulate the mirror neuron system (MNS) offers another opportunity to stimulate the damaged motor cortex before the infant has achieved volitional reach and grasp.

Theoretical framework

MNS is comprised of 'mirror neurons', specialised neurons which fire when one observes another performing an action and when one executes the action, facilitating an understanding of the action and subsequent imitation of that action.[19–22] Mirror neurons were discovered initially in the premotor area (F5) of macaque monkeys and have since been identified in the rostral area of the inferior parietal lobule (PF) and the ventral premotor cortex.[20 23] Direct evidence for MNS in humans is lacking and there have been no studies published which have recorded single neurons from the proposed MNS in humans.

There is a growing body of neurophysiological and brain-imaging studies providing indirect evidence for the existence of MNS in humans.[24] Transcranial Magnetic Stimulation studies have concluded that MNS exists in humans and it differs from MNS in monkeys.[25–29] Non-purposeful and intransitive actions activate mirror neurons in humans but not in monkeys.[26 28 30] When humans observe actions, the temporal features of cortical excitability suggest that MNS codes for the whole action as well as the individual movements that comprise the action. In contrast, only the whole action is coded by MNS in monkeys.[24] These

unique properties of MNS in humans suggest that humans' capacity to imitate others' actions is related to MNS.

Several studies have identified two cortical areas which correspond to motor function and are activated during action observation in humans: (1) the rostral area of the PF and (2) the lower area of the precental gyrus combined with the posterior area of the inferior frontal gyrus (IFG[31–45]). It has been suggested that the activation of mirror neurons located in the IFG (otherwise known as Broca's area) in humans corresponds to activation of mirror neurons located in the PF in monkeys.[24] In humans, the two mirror areas receive afferent input from the superior temporal sulcus (involved in processing motion), and send efferent input to the motor cortex.[46 47]

The functional role of MNS in monkeys as well as humans has been proposed to underlie the processes of imitation and understanding the actions of others in relation to oneself.[21 23 24 38 42 43 48–52]

Demonstration of MNS soon after birth introduces a new perspective to the treatment of infants with congenital brain injury. Emerging evidence from the basic sciences in infant rhesus macaque monkeys suggests that the immature MNS can facilitate the imitative capabilities of infants, and that consistent demonstration of imitative skills and subsequent manual skills can predict later motor development.[53 54]

Sensorimotor reorganisation after early brain injury

It is well known that brain injuries impacting on the sensorimotor (SM) system may manifest in varying degrees of functional impairment, the extent of which is related to the size and site of the lesion, as well as the type of adaptive reorganisation that follows. The main mechanism for a reconnection of the motor cortex to the spinal cord consists of reorganisation within the damaged hemisphere, based on a partial sparing of the primary motor cortex (ie, ipsilesional reorganisation). When the lesion occurs at an early stage of development, a different mechanism can also be observed, whereby a significant number of monosynaptic fast-conducting ipsilateral motor projections (from the undamaged hemisphere) persist. Such projections are normally withdrawn within the first months of life. This alternative mechanism results in the undamaged hemisphere directly controlling both upper limbs, which is a pattern of reorganisation unknown to adult pathology (ie, contralesional reorganization).[55]

Emerging evidence in humans suggests that the pattern of SM reorganisation after early brain injury is determined during the first year of life, and possibly within the first few months.[56] As children with reorganisation occurring in the damaged hemisphere (which results in the undamaged hemisphere directly controlling both upper limbs) have suboptimal upper limb motor activity, this pattern appears to be maladaptive.[55] It has been suggested that MNS may influence cortical reorganisation associated with upper limb impairment,

and could potentially be a target of very early intervention.[11]

Action observation and imitation

The process of observing an action (ie, action observation) leads to the activation of MNS and stimulates the corticospinal system (motor pathways) prior to imitating the action.[38 57–59] When the motor cortex is damaged (eg, congenital brain lesion), action observation and imitation may influence cortical reorganisation by directly restoring the damaged motor pathways or reinforcing other pathways that originally helped to perform motor actions, or both.[60]

In animal and human adult studies, action observation appears to activate MNS and enhance excitability of the SM cortex.[61] These findings suggest that the effects of an asymmetric brain lesion may be ameliorated by an infant friendly and novel upper limb rehabilitation programme based on action observation. The training programme would aim to stimulate the damaged motor pathways from the lesioned hemisphere to the impaired upper limb, which may subsequently improve later upper limb motor activity by changing the cortical reorganisation typically seen after this type of injury.

Currently available therapeutic options and limitations

Various interventions are used for improving upper limb motor function and reducing activity limitations for children with unilateral CP. A recent systematic review was conducted which evaluated all upper limb interventions for infants (<3 years) with brain injury.[61a] The interventions identified included: Constraint-Induced Movement Therapy (classic CIMT or modified for a paediatric population mCIMT); intramuscular Botulinum toxin A injections as an adjunct to occupational therapy (OT); forced-use therapy and neurodevelopmental treatment with or without upper limb casting.

The authors concluded that current evidence for very early upper limb interventions suggested small effects on unimanual capacity, bimanual coordination and self-care skills; however, there are limited data on the safety and neural mechanisms underlying activity changes in response to these interventions. Further research is required to investigate the efficacy of upper limb interventions of this at-risk population at preschool age (<3 years) and address the lack of attention to safety implications for infants.

Proposed intervention and justification: why Upper Limb Baby Early Action–observation Training?

Action–observation training (AOT) is a novel upper limb rehabilitation approach based on the recent discovery of mirror neurons.[62] This approach has been shown to effectively improve upper limb motor function in adult patients with chronic stroke.[61] AOT is currently being investigated in a population of school-aged children (5–15 years) with unilateral CP.[62]

AOT has not yet been investigated in a randomised clinical trial for a population of infants with congenital brain lesion. The efficacy, benefits and safety implications of this novel rehabilitation for this at-risk population are unknown. Ideally, such an intervention should begin soon after the brain injury has occurred. It is difficult, however, to achieve voluntary activation of the motor cortex during the first weeks of life, as voluntary reaching is absent or immature. The activation of the motor cortex related to action observation may represent a unique opportunity for therapeutic intervention in this early period of development. As soon as voluntary reaching can be reliably elicited, very early intervention should be supplemented with standard rehabilitative approaches aimed at encouraging symmetrical reach and grasp behaviours, as well as use of the limb in developing mobility.

In adults with stroke, action observation has been shown to effectively increase cortical excitability of the SM cortex and improve upper limb motor outcomes.[61] Based on the hypothesis that the same activation can be elicited in infants, we predict that action–observation training will enhance the excitability of the SM cortex and accelerate the maturation of the corticospinal tract and the shaping of the spinal motor circuits, leading to better spontaneous use of the impaired upper limb. This could prevent the development of asymmetric reach and grasp in young infants with early asymmetric brain lesions.

Studies of the early development of infants at risk of progressing to CP, such as infants born preterm, frequently include a healthy term-born reference group to take account of typical development progression. As there are very limited data on the early imitation skills of infants[63–69] or very early development of reaching and grasping[4] in both term-born and preterm infants, a parallel healthy term-born clinical trial is planned. As the provision of Action–Observation Training and Toy Observation Training (TOT) in the developmental period of 9–18 weeks post-term is considered to be low risk and developmentally appropriate training approaches, there is no risk but potentially some additional benefit for all infants. Inclusion of a cohort of healthy term born infants in a parallel randomised comparison trial will provide a typically developing comparison of training approaches to our randomised controlled trial (RCT) of infants with aBI.

Broad aim of proposed study
The broad aim of this study was to evaluate in two parallel RCTs with an identical sham control whether the novel intervention AOT is more effective than standard TOT in: (1) influencing the early development of reaching and grasping of typically developing infants (TDIs; n = 40) and (2) improving the upper limb motor activity of infants with asymmetric brain lesions (n = 32).

METHODS
Two RCTs with an identical sham control will be conducted to evaluate the efficacy of AOT compared with standard TOT in: (1) TDIs with a gestational age between 38 and 41 weeks at the time of recruitment (n = 40); and (2) infants with asymmetric brain lesions aged 0–9 post-term weeks at the time of recruitment (n = 32).

This study will involve an at-risk population of infants (ie, infants with asymmetric brain lesions). It would therefore be unethical to give no intervention to this population in the control arm. Standard TOT is the sham control intervention that will be used for both RCTs. It is similar to standard therapy as it involves the parents presenting toys to infants and encouraging spontaneous visual exploration, without demonstrating how to play with the toys. It does not include the active AOT component of the intervention for the treatment group.

The specific hypotheses to be tested are as follows:
1. TDIs receiving AOT will have faster development of reaching and grasping in both upper limbs, compared with infants receiving standard TOT.
2. Infants with asymmetric brain lesions receiving AOT will have faster development and greater quality and quantity of reaching and grasping in both upper limbs, compared with infants receiving standard TOT.
3. For both infant cohorts, AOT will result in greater equalisation of corticomotor pathways and retention of cortical reorganisation, compared with standard TOT.
4. Individual differences among infants in the quality of GMs and imitative behaviour will modulate the effects of training on their development of reaching and grasping.

These hypotheses will address the following specific aims:
1. To determine if TDIs undergoing AOT will develop reaching and grasping earlier than those undergoing standard TOT. AOT is a novel upper limb training programme based on action observation. Evidence suggests that action observation can activate the motor cortex and reinforce the corticospinal network. We will determine through an RCT if a 4-week AOT programme (from 9 to 13 post-term weeks' age) will influence the short-term outcomes of reaching and grasping, compared with a standard TOT programme whereby action observation is replaced with toy observation (no grasping action demonstrated).
2. To determine if infants with asymmetric brain lesions undergoing AOT will develop reaching and grasping earlier, and have greater quality and quantity of reaching and grasping, compared with those undergoing standard TOT. Recent studies suggest that an early therapeutic intervention in infants with aBI should aim to activate the impaired motor cortex. We will determine through an RCT if a 4-week AOT programme (from 9 to 13 post-term weeks' age) will lead to cortical activation associated with action observation and influence the development of

reaching and grasping in these infants and improve their short-term and long-term outcomes. AOT will again be compared with standard TOT. If we can show that this novel, very early intervention can improve short-term and long-term upper limb motor activity in infants with asymmetric brain lesions, this will guide clinical practice and enable a more efficient allocation of therapy resources in the future.

3. To determine if AOT will lead to greater equalisation of corticomotor pathways and cortical reorganisation. We will determine if the 4-week AOT programme will result in modified cortical coherence related to action observation through an EEG. If we can show that this novel, very early intervention can lead to greater equalisation of cortical motor pathways and retention of cortical motor reorganisation, this will guide clinical practice with implications for other patients (infants with bilateral/symmetric brain injury, school-aged children with unilateral CP, children with stroke). An understanding of the nature and timing of the brain lesion may indicate which infants respond better.

4. To determine if the individual differences among infants in the quality of GMs and imitative behaviour will modulate the effects of training on their development of reaching and grasping. We will investigate these using standardised measures of spontaneous motility and imitation skills in both cohorts, pretraining and post-training. If we find these correlations, we can explore the possibility of individually tailoring very early therapeutic interventions.

Assessments will be performed at 9, 12, 14, 16 and 18 weeks. Follow-up will be performed at 6 and 12 months following intervention, to determine the retention of effects. The timing of assessments coincides with early critical periods of spontaneous GMs and early imitation behaviours (9 weeks); period of fidgety movements (12 weeks); early symmetrical reaching (14–16 weeks) and symmetrical reaching to the midline (18 weeks) with criterion assessment on norm referenced measures at 6, 8 and 12 months CA. The experimental design and outcome measures are depicted on the CONSORT flow chart in figure 1.

The Human Research Ethics Committees at the Royal Children's Hospital, Brisbane (HREC/09/QRCH/134),
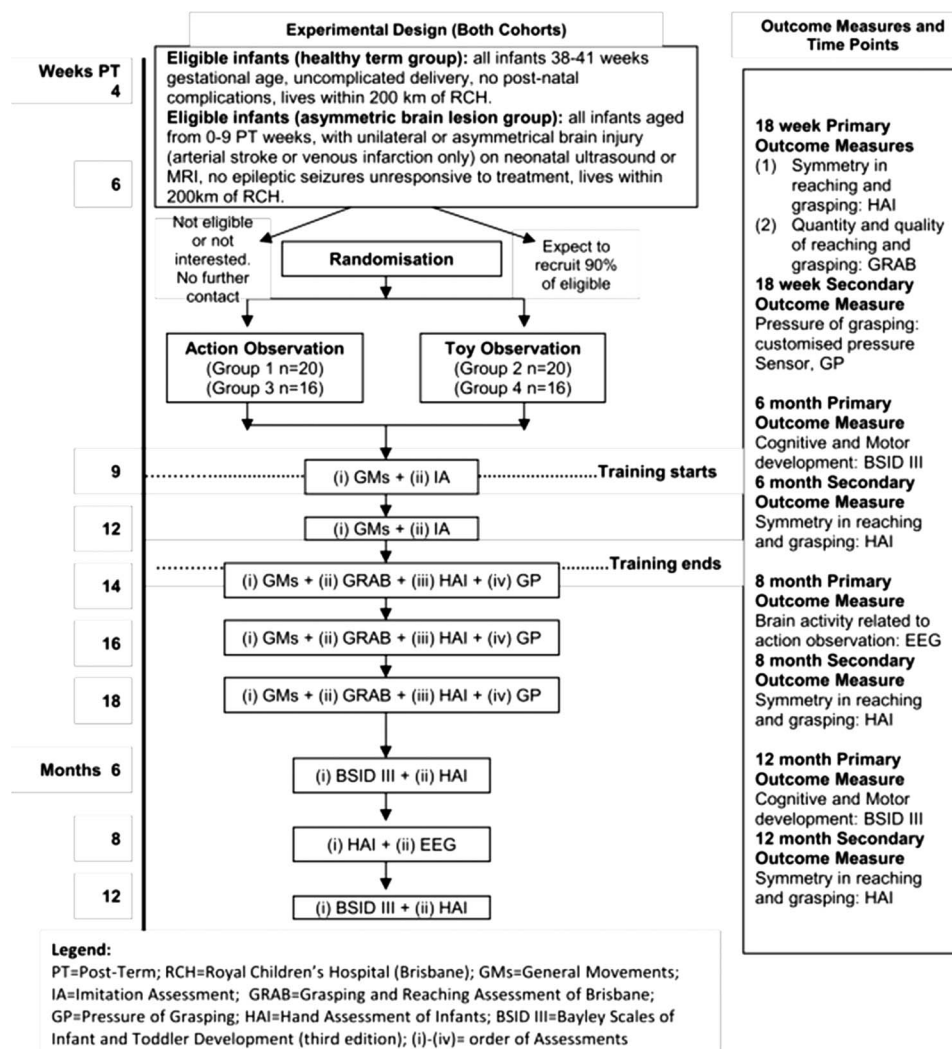

Figure 1 Flow chart of Upper Limb Baby Early Action–observation Training study according to CONSORT guidelines.

the University of Queensland (2009001870), The Royal Brisbane & Women's Hospital (HREC/09/QRCH/134), the Mater Children's Hospital and the Mater Mother's Hospital (1814MC), the Stella Maris Scientific Institute and the University of Pisa in Italy have granted approval for the study (43/2011).

Study sample and recruitment

Infants and their families will be recruited within a 50–200 km radius from The Royal Children's Hospital, Brisbane, Australia. The recruitment process will target major metropolitan health districts across southeast Queensland, with the expectation that the cohort I sample will be representative of TDIs and the cohort II sample will be representative of infants with asymmetric brain lesions from Queensland.

Recruitment has been expanded to cover a 200 km radius from the Royal Brisbane and Women's Hospital in Brisbane which includes three additional neonatal follow-up teams at the Mater Mother's Hospital, Nambour Hospital and the Gold Coast Hospital in Queensland. All regional Paediatricians, Child Neurologists, Neonatologists, rehabilitation Physicians and Allied Health professionals (Occupational Therapists, Physiotherapists) have been informed of the study and referral processes. Similar strategies for achieving adequate participant enrolment have been adopted in the region of Tuscany, in Italy.

Inclusion criteria

Cohort I: TDIs will include infants
1. With a gestational age at birth between 38 and 41 weeks;
2. Living within a 50 km radius of the Royal Children's Hospital, Brisbane.

Cohort II: Infants with aBI will include infants
1. With an asymmetric (one-sided or more involved on one side) or unilateral (one-sided) brain injury (eg, preterm or term arterial stroke, grade III or IV intraventricular haemorrhage, periventricular leukomalacia) identified on neonatal ultrasound or MRI;
2. Aged 0–9 post-term weeks at the time of recruitment;
3. Living within a 200 km radius of the Royal Children's Hospital, Brisbane.

A parallel clinical trial will admit infants with the same inclusion criteria into two parallel RCTs at the Stella Maris Scientific Institute in Pisa, Italy.

Exclusion criteria

Cohort I (TDI) will exclude infants
With any postnatal medical complications (eg, jaundice) requiring extended hospital admission or medical treatments.

Cohort II (aBI) will exclude infants:
With epileptic seizures unresponsive to treatment.

Sample size

Cohort I (TDI): Based on a two-tailed t test of two independent means, with a significance (α) level of 0.05, 80% power, predicted effect size of 0.8 and a 90% retention rate, we require 20 participants in each group (total sample of 40) for cohort I.

Cohort II (aBI): The sample size for cohort II was based on the assumption that the effect size of the proposed training will be similar to that found by Heathcock *et al*[14] in a population of preterm infants (n = 26) with a comparable training programme, with a mean effect size of 2.4. Given the high effect size, the calculation returned a sample of only four participants per group, on a two-tailed t test, with a significance (α) level of 0.05 and 80% power. As our cohort II population will consist of two subgroups of lesion type (ie, arterial stroke and venous infarction) and will be highly variable with the presence of aBI, we have quadrupled the sample. We require 16 participants in each group (total sample of 32) for cohort II.

Randomisation

The allocation sequence will be comprised of computer-generated random numbers in a blocked design. Infants will be randomised to receive either AOT or standard TOT, from concealed envelopes opened by non-study personnel. Treatment allocation will be recorded on a piece of folded paper inside each envelope in random order (computer generated). The randomisation process will involve allocating a code to each infant, which consists of the letter 'B' or 'G', according to gender, and a number based on the date of birth (eg, 'B1', 'B2' and 'G1', 'G2'). The infant's name and code will be written on the paper inside the envelope and sealed. The envelope will be marked that it has been allocated and the infant's code will be written on the front of the envelope. As each infant's code is entered, he/she will be allocated the next consecutive envelope, which will be opened by the non-study personnel who will read and record the treatment allocation from the paper inside the envelope. The randomisation envelopes will be held and administered by the therapist providing training of the interventions for the parents.

Blinding

The therapists who will be training the parents in the interventions and the parents will be informed of group allocation; the therapists conducting the assessments will be masked to group allocation; and study personnel who will be assessing the outcomes will also be masked to group allocation. Randomised group allocation will remain concealed to the therapists who conducted the assessments until all data for the entire sample have been analysed.

Study treatments

Both cohorts will receive the same dosage of three 5 min sessions (15 min/day) for 6 days/week, for 4 weeks. The total dosage of intervention will be 6 h, over a period of 28 days. After baseline screening and

randomisation, infants will receive either AOT or standard TOT.

Parents will be trained by an occupational therapist for approximately 30 min, and they will be directly observed performing the training activities with the infant during the training. Two follow-up phone calls regarding questions on how to perform the training will be addressed over the telephone with the therapist who trained the parents. Parents will be asked to video-record the sessions each day. Parents of the AOT group will repeatedly show the infant a grasping action on a set of three toys, presented in random order. Parents of the TOT group will show the infant the same set of three toys, also presented in random order, without the grasping action. The toys are mostly cylindrical in shape and vary in appearance, colour and patterns (ie, cow, clown and musical instrument). Groups will be compared at 14, 16 and 18 weeks, as well as 6 and 12 months following the intervention.

To optimise comfort and convenience for their families, the intervention training for parents, delivery of intervention and all assessments from 9 to 18 weeks will be performed in the infants' home environment. To optimise the infant's engagement in the interventions, parents will be advised to: (1) perform the training when the infant is calm and alert; (2) wiggle their fingers to engage the infant's attention prior to the start of the training and (3) stop the training and allow the infant to play briefly with the toys if the infant becomes distracted or stops attending to the parent's hand and toy, before continuing the training.

Therapy protocols and delivery

Several occupational therapists will plan and conduct both the intervention groups. These core therapists will be responsible for liaising with the parents to organise home visits to train the parents in their allocated interventions and for each set of assessments at 9, 12, 14, 16 and 18 weeks' CA. The core therapists will also be responsible for organising the 6-month and 12-month follow-up assessments at the Royal Children's Hospital, Brisbane. Two other occupational therapists will provide training and follow-up phone calls for the parents.

An online diary that will only be accessible to the core therapists will be completed after each training session, follow-up phone call, home visit and follow-up assessment to summarise each activity for each infant. Any issues of concern such as difficulties with training and adverse events will be considered when data are analysed as potential factors that may account for differences between cohorts. Video footage of each training and assessment session will be qualitatively and/or quantitatively analysed to assess treatment fidelity.

The core investigator team (RNB, JZ, AG, KP, LF and MP) will meet regularly to review the progress of training and assessments for both cohorts, and will decide when any modifications to the protocol are required. The alternate training programme (AOT and TOT) is standardised and would not be modified during the intervention period from 9 to 18 weeks post-term. There is no scientific expectation for discontinuing or modifying either the AOT or TOT, as there is no evidence to support one method over the other. Any additional interventions (motor training by Physiotherapists or Occupational Therapists will be monitored including the dose, focus and content of concomitant training) and medications (for epilepsy) will be recorded at the next home visit and accounted for in the secondary analysis. Parents in either study will be free to discontinue the training and exit the study if they wish, and there would be no impact on their access to additional medical and allied health services.

Outcome measures and procedures

At 14, 16 and 18 weeks post-term or CA, the quantity and quality of reaching and grasping will be measured using the Grasping and Reaching Assessment of Brisbane (GRAB); symmetry of reaching and grasping will be measured using the Hand Assessment of Infants (HAI) and pressure of grasping for each hand with a customised pressure sensor. At 6 months, the primary outcome measures will be HAI and BSID III, to measure cognitive and motor development. At 8 months, HAI and EEG will be used to measure brain activity and cortical coherence. At 12 months, the primary outcome measures will again be HAI and BSID III.

Grasping and Reaching Assessment of Brisbane

This is the primary outcome measure of the study, developed by the research team. GRAB will be performed at 14, 16 and 18 weeks post-term. TDIs in Western cultures have been observed to acquire the important motor skills of reaching between 3 and 5 months of age[70 71] and grasping as early as 18 weeks.[72–74] Prior to reaching onset, infants have been observed to demonstrate pre-reaching movements. These movements provide infants with multimodal input about their upper limb function within their environment, as well as with SM experiences that can help infants to learn how to control their upper limbs.[70 75–77]

Infants will be secured in a Baby Björn Babysitter Balance infant chair, allowing full range of motion of the arms. They will be presented with a toy at shoulder height at 75% of arm length for six trials of 30 s each, in the midline. Three different toys will be used in the various trials, in random order, to maintain the infant's interest in the task. A video camera will be placed to the midline at approximately 1.2 m above the infant to ensure a full view of the infant, his/her upper limbs and the toy. The video recordings will be edited into fragments in which the infant is manipulating the toys, and will be analysed by researchers who are masked to group allocations. The following variables will be assessed: (1) number of hand-toy contacts, (2) hand-toy contact type, (3) hand-toy contact duration with visual attention, (4) hand-toy contact duration without visual
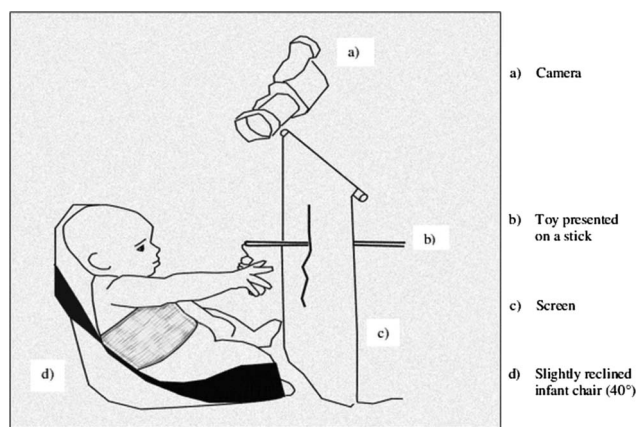
Figure 2 Schematic drawing of the setting for the Grasping and Reaching Assessment of Brisbane.

attention and (5) number of bilateral interactions. As GRAB has been developed by our team, we propose to establish validity and reliability (intrarater, inter-rater and test–retest). See figure 2 for a schematic drawing of the GRAB set-up.

Pressure of Grasping

Infants will be secured in the same infant chair used in GRAB. They will be presented with a small customised pressure sensor in the form of a cylindrically shaped toy that allows the recording of differential positive pressure. A soft foam strap is attached to the pressure sensor to secure the infant's hand. Pressure will be continuously sampled at a minimum rate of 20 Hz and stored on a PC compatible computer for further analysis. Each hand will be approached separately, using the pressure sensor. One trial will be performed for each hand, in a random order. The recording will begin as soon as the infant grasps the pressure sensor and will continue for 120 s, unless the infant drops it. In that case, the trial will be repeated. The assessment will be video-recorded, synchronising the images with the activity of the pressure sensor. Grasp pressure will be assessed by the time series of positive hand pressure (expressed in Volts) corresponding to the selected video fragments, which will be analysed. The measures extracted will be: (1) maximum pressure, (2) minimum pressure and (3) variance.

Hand Assessment of Infants

HAI is a new assessment tool which aims to quantify hand function from 2 to 8 months post-term. It will be performed at 14, 16 and 18 weeks, and again at 6 months post-term. The scale was developed at the Karolinska Institute of Stockholm (Sweden; Professor Eliasson and Professor Sundholm) in collaboration with the University of Pisa. It is currently at the phase of standardisation in a normal population. The assessment is based on a video-recorded play session, which should be completed in approximately 10 min. Upper limb movements, reaching and grasping will be elicited by

presenting the infants with toys. The toys are designed to promote exploration and handling and are presented in various places (eg, both sides, midline, close to the baby and at a distance) on multiple occasions, both from the assessor's hand and, when possible, on the table. The scale consists of 40 items and includes both unimanual and bimanual tasks. Video recordings will be assessed by a researcher who is masked to group allocations.

Prechtl's Assessment of General Movements

The assessment of GMs based on Prechtl's method of observation is largely used as a diagnostic tool for neurological evaluation of the newborn and the young infant.[78–80] GMs have shown a high predictive value for neurodevelopmental outcome at 12–24 months for at-risk infants (eg, brain lesion, CP, preterm); sensitivity is $\geq 92\%$ and specificity is $\geq 82\%$, $p < 0.01$.[81] They have greater sensitivity in predicting CP than other motor assessments used in infancy.[81] GMs involve assessing the quality of spontaneous motility using a short video recording. Video recordings will be performed at 9, 12, 14, 16 and 18 weeks' CA. Video recordings will be performed for 5 min and there will be one additional minute to focus on each hand. The video camera will be positioned in the midline approximately 1 m above the infant, at an angle of 45°. Infants will be recorded while the infant is in a calm, alert state at interfeeding time, in a supine position, and clothed with wrists and ankles exposed. The analysis of GMs will be performed by one of the certified GMs assessors participating in the study, who will be masked to group allocations.

Assessment of Imitation

All infants will be tested for simple gestural and vocal imitation on two separate occasions, pretraining and post-training, at 9 and 12 weeks post-term age. This assessment will determine whether individual infants have a reliable imitative response, which may be important in interpreting the intervention results, as individual differences between infants are expected. The 9-week time point occurs prior to the intervention; the 12-week time point occurs 1 week prior to completion of the intervention. These time points have been specifically selected to determine: (1) infants who are strong imitators; (2) gestures that are reliably imitated and (3) whether the imitative responses have been influenced by the intervention.

Infants will be assessed on the gestures most commonly reported in the neonatal imitation literature[63–69]: (1) four facial gestures: tongue poking, mouth opening, happy and sad emotional expressions; (2) two manual gestures: opening and closing of the hand (grasping action) and index finger pointing and (3) two vocal gestures: 'EEE,' 'OOO', as well as tongue clicks. The order of presentation for the gestures will be randomised across infants. The assessment will be video recorded.

A trained coder, masked to group allocations for the entire duration of the study, will score imitation from the videotapes. The coder will view the footage of the infants' behaviour during the assessment, and record frequencies for each of the aforementioned gestures. These frequencies will be interpreted relative to the gestures that were modelled. Imitation is evident when infants' production of a gesture is significantly greater in response to a matching gesture, than to any other gesture.

Bayley Scales of Infant and Toddler Development (tThird edition)

BSID III (Bayley-III Clinical Use and Interpretation, Saint Louis, Missouri, USA: Elsevier Science & Technology, 2010) will be performed at 6 and 12 months CA. These time points were chosen as: (1) reaching and grasping are expected to be established by 6 months of age; (2) bimanual manipulation is expected to be established by 12 months of age. BSID III will be used to assess cognitive and motor development. It is a frequently used standardised developmental assessment throughout Australia; however, its clinical utility in various populations of children has not yet been established.[82] It will consist of a series of simple interactions with the infant and will take between 50 and 80 min to administer.

Mean reliability coefficients were: 0.91 (Cognitive composite scale), 0.86 (Fine Motor subtest), 0.91 (Gross Motor subtest).[83] Corrected correlation coefficients for test–retest reliability were: 0.67 (Fine Motor subtest, 2–4 months) and 0.83 (Gross Motor subtest, 33–42 months).[83] Correlation between the BSID III Cognitive composite score and BSID II Mental Index score was 0.60; correlation between the BSID III and BSID II Motor composite scores was also 0.60.[83] High correlations were found between the Wechsler Preschool and Primary Scale of Intelligence (third edition) Verbal, Performance and Full-Scale scores and the BSID III Cognitive score (0.72–0.79).[83] Moderate correlations were found between the BSID III Motor composite and the Peabody Developmental Motor Skills (second edition) Motor quotients (0.49–0.57).[83]

Electroencephalogram

This test will be performed at 8 months post-term and will last approximately 25 min. EEG is a standard method used in infants to measure brain activity and will be used in this study to explore possible brain functional correlates of motor development. It demonstrates mu rhythm suppression, which is considered to be a possible index of mirror neuron activity during the observation and execution of hand actions.[84 85] We will use a certified advanced system that is extensively used in infant testing, known as the Geodesic Sensor Net. It consists of a high-density net, which is applied in a few seconds.

We have tested modifications of mu rhythm using independent component analysis (ICA) of high-density EEG recordings, according to the paradigm used by Nyström et al.[86] ICA is a blind source separation technique that aims to find components that are most statistically independent of each other. The mu rhythm is expected to decompose into one or a few components from each subject, and these are the only components useful for the analysis. We will have three different conditions for the infants to perform, from which mu rhythm activation will be estimated: (1) observe a static human model (baseline); (2) move his/her hand by reaching for and grasping an object (goal-directed action) and (3) move his/her hand by placing it on the table (non-goal-directed action). The difference in mu rhythm activation between the baseline and the two movement conditions will be used for the selection of EEG sources, and the difference between the two movement conditions will be analysed.

Analyses

Analyses will be conducted on an intention-to-treat basis using STATA 11. Data from each outcome measure will be summarised for each treatment group and descriptive statistics (frequencies, means, medians, 95% CIs) calculated dependent on data distribution. A significance level of 0.05 will be used. The effects of AOT on development of reaching and grasping (hypotheses 1–3) will be explored by a two-way repeated measures analysis of variance for parametric variables, including duration of hand-toy contact, maximum and minimum pressure and pressure variance. Correction for multiple comparisons will be applied. The Kruskal-Wallis statistic will be used for non-parametric measures, including the number of hand-toy contact and the IHA score. To test the possible influence of GMs quality and imitative behaviour (hypothesis 4), these will be considered as covariates in a multifactorial analysis. The results of EEG signal analysis will be compared between the two groups of each cohort using parametric tests. Post hoc analyses will be undertaken to investigate the clinical characteristics of infants who have a greater response to either intervention.

DISCUSSION

This paper outlines the background and design for two parallel RCTs with an identical sham control, comparing AOT with standard TOT to: (1) influence the early development of reaching and grasping of TDIs and (2) improve the upper limb motor activity of infants with asymmetric brain lesions. To our knowledge, this study is the first to directly compare the two approaches for this population. Furthermore, we will be establishing validity and reliability for the newly developed outcome measures.

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