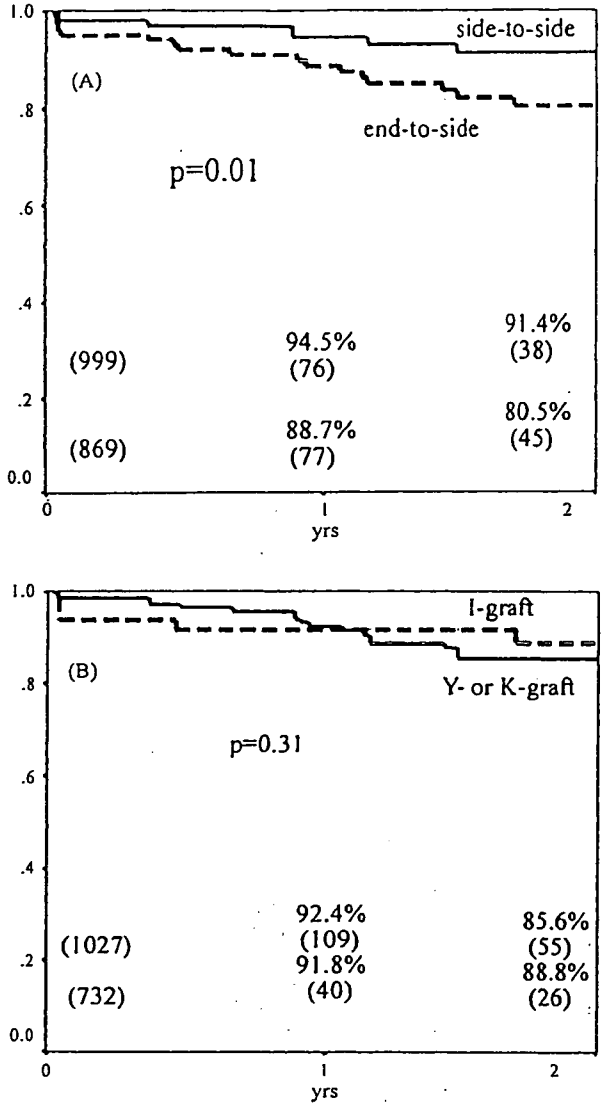


Fig. 3. (A) The actuarial graft patency rate of the bypass grafts to the non-LAD branches. End-to-side anastomoses (graft end) vs side-to-side anastomoses. (B) The actuarial graft patency rate of the bypass grafts to the non-LAD branches. I-graft vs Y- or K-graft.

sufficient antegrade flow to the LAD territory, even with moderate stenosis.

For the coronary branches besides LAD, there was no obvious disadvantage of the composite grafts versus the individual graft and the in-situ ITA. In addition, native coronary stenosis had stronger impact on the bypass grafts to the non-LAD branches than on the bypass grafts to the LAD in the follow-up angiographic results. For the bypass grafts to the non-LAD regions, both grade B/C and 51–75% stenosis in the native coronary branch significantly correlated with graft occlusion.

One of the possible explanations for these differences between the grafts to LAD and those to non-LAD branches may be the difference in the graft materials. About 90% of the anastomoses were performed with the composite radial artery. The radial artery may be more sensitive for the blood flow in the lumen than the ITA graft. More severe stenosis will be necessary for the long-term patency of the radial artery, as compared with the ITA graft.

Regarding the conduit type, no significant difference was found between the I-graft and the Y- or K-graft in the non-LAD regions. We consider that the appropriate pressure slope in each segment of the bypass conduit, highest at the proximal and lowest at the end of the conduit, was the most important for antegrade bypass flow to all target vessels. The bypass grafts with the side-to-side anastomoses presented better graft patency than those with the end-to-side anastomoses. Therefore, when the positional relationship of the target sites allows, the I-graft would be favorable, because it has only one end-to-side anastomosis and the target coronary branch at the end of the conduit can be changed by simply determining its orientation. On the other hand, the Y-graft has the advantages of increased flow capacity [15] and availability to the distant target branches.

Dion et al. reported that the patency rate of end-to-end grafting was comparable with side-to-side grafting with excellent long-term patency of sequential grafting using the ITA graft [14]. In their report, the target branches of 78% of bypass graft restudied were the LAD and a diagonal branch, whereas, in the present study, sequential ITA grafting to the LAD and a diagonal branch was only 9%, and sequential grafting to four or more target branches was performed in about 11% of patients. We consider that the difference is owing to differences in target site, graft material, and probably the number of target coronaries in sequential anastomoses.

Selection of patients suitable for this procedure would be a next concern. It has been widely accepted that the patients with severe atherosclerosis of the ascending aorta are the most suitable candidates for composite and sequential grafting [10,11]. We would suggest herein new patients' selection criteria from a viewpoint of preventing competitive flow and maximizing durability of arterial grafts. According to the results of the present study and our previous investigations, the decisive risks of competitive and reverse flow are as follows: (1) a RCA branch with 51–75% stenosis, (2) a LCX branch with 51–75% stenosis, (3) a bypass conduit with four or more distal anastomoses, and (4) three high-risk situations reported in [9]. Of 677 patients in this study, 147 (21.7%) patients had none of these risks and/or all risky situations were successfully avoided. The actuarial graft patency rate of patients who have none of the above risks at 3 years was significantly higher than that of patients with any of the risks (92.6% vs 69.7%; $p < 0.0001$). They were the best candidates for this procedure. On the other hand, when competitive or reverse flow is highly predicted, alternative strategies, such as the aortocoronary bypass, which provides the highest bypass pressure [16], may be reasonable, especially for the non-LAD regions.

The present study had some limitations. First, the patients who underwent follow-up angiography were biased toward clinically evident graft failure. Second, the peripheral vascular resistance in the myocardial tissue, which has an important role in the coronary perfusion, was not taken into account. Third, the capacity of the ITA graft was not considered. The pressure and flow capacity as the blood source of the bypass conduit and potentiality of growth or thinning and adaptability to the graft flow may also play important roles in the occurrence of insufficient flow and resultant occlusion. At the beginning of 2004, we started to harvest ITA in a skeletonized fashion to maximize the

Table 4
Predictors of graft occlusion in the intermediate-term follow-up period

| Variables | Hazard ratio | 95% CI | p-Value |
|---|---|---|---|
| Univariate analysis | | | |
| Female | 1.17 | (0.55–2.47) | 0.68 |
| Distal anastomoses of the conduit | 1.00 | (0.78–1.29) | 0.99 |
| Early period (Dec. 2000–Feb. 2003) | 0.91 | (0.50–1.66) | 0.76 |
| Type of the conduit, Y- or K-graft (vs in-situ ITA) | 0.66 | (0.31–1.42) | 0.29 |
| Type of the conduit, I-graft (vs in-situ ITA) | 1.02 | (0.45–2.32) | 0.96 |
| Graft material anastomosed, radial artery (vs in-situ ITA) | 1.39 | (0.81–2.38) | 0.23 |
| Graft material anastomosed, free ITA (vs in-situ ITA) | 2.51 | (0.74–8.55) | 0.14 |
| Location, LCX territory (vs LAD territory) | 0.98 | (0.51–1.90) | 0.95 |
| Location, RCA territory (vs LAD territory) | 2.44 | (1.41–4.23) | 0.001 |
| Stenosis (51–75%) | 2.28 | (1.35–3.83) | 0.002 |
| Diameter of coronary branch (<1.5 mm) | 1.94 | (1.12–3.36) | 0.01 |
| End-to-side anast. (graft end) (vs side-to-side = sequential proximal) | 1.48 | (0.87–2.53) | 0.15 |
| Grade B/C in early angiography | 6.46 | (3.64–11.47) | <0.0001 |
| Multivariate analysis | | | |
| Graft material anastomosed, radial artery (vs in-situ ITA) | 0.51 | (0.10–2.70) | 0.43 |
| Graft material anastomosed, free ITA (vs in-situ ITA) | 1.24 | (0.14–11.35) | 0.84 |
| Location, LCX territory (vs LAD territory) | 1.88 | (0.37–9.57) | 0.45 |
| Location, RCA territory (vs LAD territory) | 3.27 | (0.65–16.35) | 0.15 |
| Stenosis (51–75%) | 2.86 | (1.17–6.99) | 0.02 |
| Diameter of coronary branch (<1.5 mm) | 1.57 | (0.78–3.14) | 0.20 |
| End-to-side anast. (graft end) (vs side-to-side = sequential proximal) | 1.12 | (0.53–2.33) | 0.77 |
| Grade B/C in early angiography | 4.19 | (2.02–8.69) | <0.0001 |

CI, confidence interval; LAD, left anterior descending artery; LCX, left circumflex artery; RCA, right coronary artery; ITA, internal thoracic artery; RA, radial artery.

capacity of the in-situ ITA graft [17,18]. This technique will extend the application of the bilateral ITA grafting to patients with a substantial operative risk [19]. Fourth, the effects of the luminal size of arterial conduits on the long-term patency remain unclear. Previously, the grading system of the luminal size at the narrowest portion, and intimal irregularity was reported [20,21]. It was reported useful for assessment of degeneration of bypass grafts in a conventional technique. However, the luminal size of the side-to-side anastomosis in the sequential fashion is not precisely measurable, especially when the angle between the graft and the coronary branch is near 90 degrees, or when the contrast medium only fills incompletely due to mixture with the blood flow from the native coronary artery. Moreover, the regression of stenosis and the increase of the diameter were relatively common findings in the arterial materials [22,23]. At last, high-pressure injection of contrast medium may induce reverse and competitive flow and may interfere with evaluation of graft flow direction. This may be a methodological limitation. This flow grading system is not necessarily practical for postoperative evaluation for each patient and each bypass graft. In the present study, flow grading was performed independently from the catheterization team. We utilized this grading system for comparison of graft configurations and optimizing the strategy for design of the arterial grafts, based on data of a considerable number of patients and bypass grafts, and examined significance of correlations between characteristics of the bypass grafts and the occurrence of competitive and reverse flow. For these purposes, flow grading is considered useful.

In conclusion, prediction and prevention of competitive and reverse flow may be necessary to enhance the advantage of multivessel revascularization using exclusively arterial materials because insufficiency of the antegrade flow would spoil the advantage of arterial grafts.

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OBSTETRICS

# A framework for standardized management of intrapartum fetal heart rate patterns

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**OBJECTIVE:** The purpose of this study was to classify fetal heart rate (FHR) monitor patterns according to risk of fetal acidemia and risk of evolution to a more serious pattern and to use this information to construct a standardized process for FHR pattern management, with the ultimate aim of minimizing newborn infant acidemia without excessive obstetric intervention.

**STUDY DESIGN:** We have identified 134 FHR patterns that have been classified by baseline rate, baseline variability, and type of deceleration. Based on the best available evidence, we have assigned a risk of newborn infant acidemia or low 5-minute Apgar score to these patterns. We have also evaluated each pattern for the risk that the pattern would evolve further into a pattern with a higher risk of acidemia.

**RESULTS:** Each FHR pattern has been color-coded, from no threat of fetal acidemia (green, no intervention required) to severe threat of acidemia (red, rapid delivery recommended). Three intermediate categories (blue, yellow, and orange) require escalated informing of appropriate individuals for intervention and resuscitation (obstetrician, anesthesiologist, and neonatal resuscitator) and preparation for urgent delivery (eg, staff and surgical suite availability and conservative techniques to ameliorate the FHR patterns).

**CONCLUSION:** This framework is applicable potentially to the institutions where it was developed and will need to be modified for other situations, depending on the logistics, facilities, and personnel available. This may provide a framework for developing algorithms for the standardized management of FHR patterns during labor, which can be tested for validity.

**Key words:** fetal acidemia, fetal heart rate management, intrapartum

**Cite this article as: Parer JT, Ikeda T. A framework for standardized management of intrapartum fetal heart rate patterns. Am J Obstet Gynecol 2007;197:26.e1-26.e6.**

Despite numerous attempts in the past 30 years, the obstetric community has been unable to reach a broad consensus on a standardized approach to the management of most fetal heart rate (FHR) monitoring patterns. Such disagreement can be seen in the National Institute for Child Health and Human Development (NICHD) publication regarding FHR nomenclature, which contained a small clinical statement.[1] There was consensus that the normal pattern (defined as normal baseline rate, normal [moderate] FHR variability [FHRV], presence of accelerations, and absence of decelerations) confers an extremely high predictability of a normally oxygenated fetus when it is obtained. Thus, no intervention is required for this pattern. At the other end of the spectrum from normality, there was consensus that the pattern of recurrent late or variable decelerations or substantial bradycardia, with absent FHRV, is predictive of current or impending fetal asphyxia so severe that the fetus is at risk of neurologic or other fetal damage or death. The implication is that the fetus should be delivered as soon as possible, unless acidemia can be ruled out rapidly.

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Received Nov. 6, 2006; accepted Mar. 12, 2007.

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0002-9378/$32.00

doi: 10.1016/j.ajog.2007.03.037

Despite the consensus regarding these 2 patterns, the members of the NICHD committee were unable to make overall recommendations for the FHR tracings between these 2 extremes, which represent at least 50% of all intrapartum fetuses, because of the uncertainty in our current state of knowledge about the presumed condition of the fetus in such cases.

The Royal College of Obstetricians and Gynecologists (RCOG) Clinical Effectiveness Support Unit[2] issued a substantial document in 2001 on the use of electronic fetal monitoring, which apparently expanded the guidelines that were proposed by the International Federation of Gynecology and Obstetrics (FIGO) in the 1980s[3] and comprehensively examined the world's literature on the subject. In that document, they classified patterns as normal, suspicious, or pathologic, depending on the incidence of 4 "nonreassuring" or "abnormal" characteristics of the FHR pattern, which they have defined. The guidelines recommended conservative or ameliorating techniques for the suspicious (1 FHR abnormality) categories. For the pathologic categories (≥2 FHR abnormalities) conservative means plus fetal blood sampling are recommended; if fetal blood sampling is not possible, then delivery should be expedited.

The American College of Obstetricians and Gynecologists (ACOG) re-

cently reissued a Practice Bulletin on Intrapartum Fetal Heart Rate Monitoring.[4] Although the preamble purports to describe the management of nonreassuring FHR patterns, the body of the text is concerned mainly with tracing assessment, ancillary testing to rule out acidemia or hypoxia, and "intrauterine resuscitation." The latter techniques are used to ameliorate FHR patterns that are presumed to represent fetal jeopardy.

Although these and other guidelines may be of some use, we have found them to be of limited use in our own labor and delivery room setting. For example, the 4 "abnormalities" of FHR in the RCOG[2] document are neither universally accepted nor equally weighted for degree of risk of fetal jeopardy. Again, fetal blood sampling, which is an important aspect of the RCOG guidelines, is used rarely in the United States today. Fetal stimulation testing is not part of the guidelines. Many of the previously recommended approaches have omitted reference to the likelihood of patterns that evolve to more severe types and have lacked recommendations regarding the speed of clinical reactions to certain more serious patterns to minimize fetal acidemia.

Despite these official positions, we believe that, because of the ubiquity of FHR monitoring, there is an urgent need to standardize management more specifically at this time, with the use of the best available evidence.

In an attempt to develop practical guidelines for the intermediate patterns mentioned in the NICHD document,[1] a multidisciplinary committee at the University of California, San Francisco, produced a 90-page document for the management of all conceivable FHR patterns for internal use. Our intramural committee attempted to determine the severity of FHR patterns that were based on the risk of fetal acidemia by reference to evidence in the literature.[5] This formed the basis for the management recommendations. However after a period of having it available to staff on the labor and delivery unit, we found that is it was infrequently used because of its complexity.

From this vantage point, we now have developed a set of algorithms and recommendations that are much simpler in presentation and therefore may be of more usefulness in practice. As before, the algorithms and recommendations are based on the best available evidence regarding the risk of acidemia of the various patterns, and we have incorporated probability of evolution to more serious patterns as an indicator of urgency of preparation for delivery.

We must stress that this approach was developed in institutions with specific logistics, facilities, and staffing and is highly unlikely to be applicable to other institutions without modification. In addition, although it has been used in our units to demonstrate feasibility, it has not been subjected to appropriate prospective testing, which must be done to determine its validity.

## Material and Methods

We constructed a grid of all possible heart rate patterns based on baseline rate (normal, tachycardia, and bradycardia), type of decelerations (early, late, variable, and prolonged), and quantity of variability (undetectable, minimal, moderate, and marked). All definitions were according to the NICHD statement on the nomenclature of FHR patterns.[1] In defining the degree of severity of decelerations, we used the classifications of Kubli et al,[6] in some cases with slight modifications.

*Variable decelerations* were defined by the National Institutes of Health (NIH) guidelines, and we used the diagram proposed by Chao[7] to quantify them. Severe variable decelerations are ≥60 seconds in duration and <70 beats/min or ≥2 minutes in duration and <80 beats/min. Moderate variable decelerations have a duration of 30 to 60 seconds and are <70 beats/min or ≥60 seconds in duration and <80 beats/min. All other variable decelerations are mild. An unresolved feature of this quantitation is whether the FHR must be below the minimum specified FHR for the whole of the specified time. We have decided arbitrarily that the FHR deceleration must be below this minimum for at least 10 seconds.

*Late decelerations*, as defined by NIH guidelines, are severe if the decrement of the deceleration is ≥45 beats/min below the baseline, moderate if the decrement is >15 beats/min but <45 beats/min below the baseline, and mild if the decrement is no more than 15 beats/min below the baseline.

Early decelerations were not quantitated because of their rarity and disagreement about the definition in the past.

*Prolonged decelerations*, as defined by NIH guidelines, require the FHR to be depressed for '2 minutes. *Severe* was defined as <70 beats/min, moderate as between 70 and 80 beats/min, and mild as not <80 beats/min. These are criteria that are similar to those used for quantitating bradycardias.

We initially evaluated each of the patterns on the basis of the risk of fetal acidemia. These associations were made on the basis of a survey of the literature that related FHR patterns to the likelihood of acidemia.[5] The following conclusions were drawn from these associations: (1) The presence of moderate FHRV, even in the presence of decelerations, is associated strongly (98%) with the absence of pH ≤7.15 or Apgar score of <7 at 5 minutes. (2) Minimal or less FHRV with decelerations has a 23% association with pH <7.15 or Apgar score of <7 at 5 min-

**TABLE 1**
**Five gradations of fetal acidemia**

| Category | Definition |
|---|---|
| Green | No acidemia |
| Blue | No central fetal acidemia (oxygenation) |
| Yellow | No central fetal acidemia, but FHR pattern suggests intermittent reductions in $O_2$ which may result in fetal $O_2$ debt |
| Orange | Fetus potentially on verge of decompensation |
| Red | Evidence of actual or impending damaging fetal asphyxia |

**TABLE 2**
**Risk of acidemia, evolution of FHR patterns to more serious risk, and recommended action**

| Variable | Risk of acidemia | Risk of evolution | Action |
|---|---|---|---|
| Green | 0 | Very low | None |
| Blue | 0 | Low | Conservative techniques* & begin preparation |
| Yellow | 0 | Moderate | Conservative techniques* & increased surveillance |
| Orange | Borderline/acceptably low | High | Conservative techniques* & prepare for urgent delivery |
| Red | Unacceptably high | Not a consideration | Deliver |

* See Table 3.

utes. (3) The likelihood of acidemia increases with the depth of decelerations, especially with late decelerations, and particularly in patterns with reduced FHRV and more so with absent variability. The risk categories depend on decelerations being recurrent (that is, occurring with ≥50% of contractions in any 20-minute segment).[1]

We then evaluated the risk that the patterns would evolve into a more serious pattern with a higher risk of acidemia. This was based on a conclusion from the previously mentioned report,[5] that, in a fetus with a pattern evolving from normal to decelerative with reduced FHRV, potentially hazardous acidemia develops relatively slowly, over a period of ≥1 hour. It was also based on preliminary work that showed the evolution of patterns in a consecutive series of >1000 fetuses in the last hour before delivery.[8]

Each pattern was classified into 1 of 5 categories for risk of acidemia and evolution to more serious patterns. Other proposed FHR management systems have used 5 categories of risk of either fetal acidemia or hypoxia.[9,10] We made use of the color coding of the Homeland Security Advisory System[11] for the risk of a terrorist attack by categorizing the risk from green (low risk) to red (severe risk). We have substituted the risk of fetal acidemia in these color-coded groups (Table 1).

In place of the protective measures that were proposed by the Homeland Security Advisory System, we have substituted protective measures to avoid acidemia in the fetus. These include a gradation of increasing surveillance and techniques for the amelioration of variant FHR patterns through the various risk groups, with the ultimate protective measure being emergency delivery.

We have not included fetal blood sampling in the management of patterns, because it is rarely used in the United States now; it has been replaced, in general, by observation of the retention of FHRV and accelerations and the use of fetal stimulation testing.

## RESULTS

A comparison of the 5 grades of the threat of fetal acidemia and evolution of the pattern is depicted in Table 2; the proposed general actions for each category are shown. The protective measures range from simple observation without intervention for the lowest risk category to emergency operative delivery for the highest risk category. The 3 intermediate categories include such actions as attempts to ameliorate the patterns with conservative techniques (Table 3).

More detailed proposed management and preparations to ensure the ability to mount a rapid response if needed and the availability of appropriate personnel are shown in Table 4.

A grid of each of the possible 134 patterns is shown in Table 5. Each pattern has been color-coded to correspond to 1 of the 5 risk categories; the categories are stratified by quantity of FHRV. In addition, 2 separate categories that are marked variability and sinusoidal patterns are appended.

The need to rule out acidemia by stimulation testing is restricted to relatively few patterns, virtually only those in which there is reduced (or sometimes absent) FHRV and the hope for a vaginal delivery in the near future. Thus, we would accept fetal stimulation testing (either tactile or vibroacoustic stimulation) as appropriate in certain cases of the fourth category (orange) or for uncertain or puzzling patterns.

## COMMENT

As noted earlier, few publications on the management of FHR patterns specify what interventions should be applied to specific FHR patterns and particularly what interventions are required to deliver a fetus in a timely fashion to avoid continuing intrauterine hypoxia. This framework has been developed to be a first step in guidelines for optimal FHR pattern management.

The proposed framework has several potential advantages over previous systems. For example the FIGO[3] and RCOG[2] approaches advise action for certain patterns that contain FHR characteristics for which there is not universal agreement regarding immediate fetal

**TABLE 3**
**Conservative ameliorating techniques for the modification of variant FHR patterns**

| |
|---|
| Position change |
| Hyperoxia |
| Correct hypotension |
| Adequate intravascular volume |
| Correct excessive contractions (eg, decrease oxytocin) |
| Avoid constant pushing |
| Tocolysis |
| Amnioinfusion to correct amniotic fluid deficit |

**TABLE 4**
**Proposed management of the color-coded categories**

| Category | Conservative techniques | Operating room | Obstetrician | Anesthetist | Newborn infant resuscitator | Location of patient |
|---|---|---|---|---|---|---|
| **Green** | No | — | — | — | — | — |
| **Blue** | Yes | Available | Informed | — | — | — |
| **Yellow** | Yes | Available | At bedside | Informed | Informed | — |
| **Orange** | Yes | Immediately available | At bedside | Present | Immediately available | Operating room |
| **Red** | Yes | Open | At bedside | Present | Present | Operating room |

jeopardy. The current proposal allows more selective approaches to each individual FHR pattern and still gives guidelines to the risk of fetal acidemia and rapidity with which preparations for delivery should be made based on the likelihood of evolution of the pattern to a pattern with a higher risk of acidemia.

The proposals in the system of Keith et al[9] have the benefit of having been subjected to validation is a nonrandomized trial and appear to minimize fetal acidemia, while also minimizing unnecessary obstetric intervention. However, the program requires special equipment that is not yet available to the practitioner.

Further ancillary testing has been proposed recently for patterns in which it is believed that the risk of acidemia is uncertain (eg, fetal pulse oximetry[12] and ST-segment analysis[13]). Pulse oximetry has not achieved acceptance as an ancillary technique to FHR monitoring in the United States because of unclear results of efficacy in trials.[14] ST-segment analysis in association with FHR monitoring has been tested widely in Europe, and trials have shown a reduction in newborn infant acidemia and no adverse effect on obstetric interventions.[13] It has been ap-

**TABLE 5**
**Risk categories for fetal acidemia related to FHRV, baseline rate, and presence of recurrent decelerations**

| Variable | No | Early | Mild VD | Moderate VD | Severe VD | Mild LD | Moderate LD | Severe LD | Mild PD | Moderate PD | Severe PD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Moderate (normal) variability** | | | | | | | | | | | |
| Tachycardia | B | B | B | Y | O | Y | Y | O | Y | Y | O |
| Normal | G | G | G | B | Y | B | Y | Y | Y | Y | O |
| Mild bradycardia | Y | Y | Y | Y | O | Y | Y | O | Y | Y | O |
| Moderate bradycardia | Y | Y | | | O | | O | O | | | O |
| Severe bradycardia | O | O | | | O | | | O | | | O |
| **Minimal variability** | | | | | | | | | | | |
| Tachycardia | B | Y | Y | O | O | O | O | R | O | O | O |
| Normal | B | B | Y | O | O | O | O | R | O | O | R |
| Mild bradycardia | O | O | R | R | R | R | R | R | R | R | R |
| Moderate bradycardia | O | O | | | R | | R | R | | | R |
| Severe bradycardia | R | R | | | R | | | R | | | R |
| **Absent variability** | | | | | | | | | | | |
| Tachycardia | R | R | R | R | R | R | R | R | R | R | R |
| Normal | O | R | R | R | R | R | R | R | R | R | R |
| Mild bradycardia | R | R | R | R | R | R | R | R | R | R | R |
| Moderate bradycardia | R | R | | | R | | R | R | | | R |
| Severe bradycardia | R | R | | | R | | | R | | | R |
| Sinusoidal | | | | | | | R | | | | |
| Marked variability | | | | | | | Y | | | | |

*B*, blue; *G*, green; *LD*, late decelerations; *O*, orange; *PD*, prolonged decelerations; *R*, red; *VD*, variable decelerations; *Y*, yellow.

**TABLE 6**
**Fetal pH in late decelerations with decreased FHRV**

| Late decelerations | Mean pH | 1 SD | 2 SD |
|---|---|---|---|
| Mild | 7.23 | 7.18 | 7.13 |
| Moderate | 7.16 | 7.12 | 7.07 |
| Severe | 7.09 | 7.04 | 6.99 |

Adapted from Paul RH, Suidan AK, Yeh S, Schifrin BS, Hon EH. Clinical fetal monitoring: VII, the evaluation and significance of intrapartum baseline FHR variability. Am J Obstet Gynecol 1975;123:206-10 (with permission).

proved recently by the Food and Drug Administration for marketing in the United States.

We believe this proposed standardization of management is required even while awaiting agreement on the acceptability of these ancillary techniques, because of the relatively long delay in the widespread introduction of these techniques. If the ancillary techniques are finally accepted, they will fit readily into these management approaches.

The ACOG[4] proposal rightly points out the relative paucity of objectively collected data for many aspects of FHR monitoring and interpretation and does not really give specific recommendations for actual management but rather gives the range of options that are currently acceptable. The ACOG guideline is quite general in many ways and of limited use to practitioners who seek specific guidance.

A number of aspects of FHR pattern management have been omitted from this framework, primarily to maintain simplicity. Our assumption is that reduced variability in the absence of decelerations is not due to hypoxia. Periods of reduced variability (eg, because of fetal sleep cycles) may last over an hour. A further point is that, in the setting of reduced variability, the presence of accelerations of the FHR (either provoked or spontaneous) gives assurance of absence of significant fetal acidemia.

A further omission from the proposal is any distinction between FHR patterns in the first and second stages of labor. Decelerations are more common in the second stage, and management in this stage is often modified by the fact that delivery may be achieved by an operative vaginal delivery, instead of a cesarean section.

In the construction of the color-coded grid, certain decisions had to be made with regard to the risk of fetal jeopardy. As noted earlier, there is good evidence that the normal trace confers a high chance of the absence of fetal acidemia and that other patterns (eg, the absence of FHRV and deep decelerations) are associated with an unacceptably high risk of acidemia. However, the many patterns between these 2 extremes have varying risks, for which there are limited data in the literature. Even where we do have data, there is still the need to make a decision regarding what level of risk is acceptable. We have used lower limit thresholds of pH 7.1 and base excess of −12 mEq/L in umbilical arterial blood as acceptable. These are 2.5% or 2 SD below the mean for normal newborn infants[15] and are well above the values in cases in which fetal hypoxic damage is seen.[16]

An example of the decision-making process in the application of risk to various patterns can be seen by reference to the categories of severity of late decelerations with reduced or absent FHRV. Data from the paper by Paul et al[17] have been abstracted from their figure that relates to fetal scalp blood pH to severity of late decelerations and are shown in Table 6. Mean values are given together with estimated SDs below the mean.

Severe late decelerations with reduced FHRV have mean pH below our threshold of 7.1 and warrant expeditious delivery. Moderate late decelerations with reduced FHRV have an acceptable mean pH, but in this category 2.5% of fetuses will have a pH <7.07, which is below our acceptable range. The 7.1 threshold lies between 1 and 2 SDs and represents perhaps 10% of fetuses in this category. Therefore, a decision must be made whether to expedite delivery in all 100% of these cases to prevent unacceptable acidemia in the 10%.

Mild decelerations with reduced FHRV present a more difficult quandary. Fetuses are 97% likely to have a pH >7.13. However, there will be approximately 1% of fetuses below our pH threshold of 7.1. Should we expeditiously deliver all 100% of these babies for the 1% who actually need it?

There is obstetric precedent for acceptable risk. For example, we offer amniocentesis for karyotyping in mothers where the risk of aneuploidy is <1%. The morbidity for well-managed vaginal breech delivery is <1%, yet patients most now have cesarean delivery. The risk of uterine rupture in vaginal birth after cesarean candidates is approximately 0.5%, but vaginal birth after cesarean birth is fast disappearing. With this in mind, we tentatively propose that a threshold risk of pH 7.1 be set to capture all but 1% of babies; we believe most of these in tracings with reduced FHRV with pH <7.1 will be relatively close to this value and >7.0.

It should be clear that the guidelines must be modified for use in institutions other than our own and may need to be modified at different times of the day, as logistics change. It should also be obvious that this is a preliminary approach, which, although it may appear to work in principle, will need to be subjected ultimately to appropriate testing. ■

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Tohoku J. Exp. Med., 2007, 212, 151-157

# Prenatal Exposure to 3,3′,4,4′,5-Pentachlorobiphenyl (PCB126) Promotes Anxiogenic Behavior in Rats

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ORITO, K., GOTANDA, N., MURAKAMI, M., IKEDA, T., EGASHIRA, N., MISHIMA, K. and FUJIWARA, M. *Prenatal Exposure to 3,3′,4,4′,5-Pentachlorobiphenyl (PCB126) Promotes Anxiogenic Behavior in Rats.* Tohoku J. Exp. Med., 2007, **212** (2), 151-157 —— Polychlorinated biphenyls (PCBs) are environmental contaminants that have adverse effects on the endocrine and nervous systems. As they are still detected in breast milk and adipose tissue in humans, the accumulated PCBs may transfer from mothers to children and damage central nervous system. It is revealed from epidemiological studies that cognitive and motor functions were damaged in children born to mothers who ingested PCBs-contaminated foods. However, it remains unclear whether prenatal exposure to PCBs affects emotionality. In the present study, we therefore examined the effect of prenatal exposure to 3,3′,4,4′,5-pentachlorobiphenyl (PCB126) on emotionality in rats by focusing on anxiogenic behavior and response of the hypothalamus-pituitary-adrenal axis to stress. Pregnant rats were treated orally with PCB126 at a dose of 30 $\mu$g/kg or corn oil, its vehicle, on gestational day 15, and their male offspring were subjected to the following experiments at 4-5 weeks old. In an open field test, rats with prenatal exposure to PCB126 showed anxiogenic behavioral responses, including decrease in time spent in the center of an open field and the number of rearings and extension of grooming duration. Interactive behavior, which is an index of anxiety level, was shortened in the social interaction test. The increase in the serum corticosterone level induced by forced swim stress was facilitated by prenatal exposure to PCB126. This evidence suggests that PCB126 may exert anxiogenicity on the offspring of exposed dams, and dysfunction of the hypothalamus-pituitary-adrenal axis may at least in part contribute to this abnormality. ——— 3,3′,4,4′,5-pentachlorobiphenyl (PCB126); prenatal exposure; anxiogenic; corticosterone


Polychlorinated biphenyls (PCBs) are widespread persistent environmental contaminants. Because of their stability and lipophilic properties, PCBs are accumulated by biological magnification; indeed, residues of PCBs have been detected in humans, fish, and wildlife (Kalantzi et

Received March 20, 2007; revision accepted for publication April 23, 2007.
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al. 2004; Carlson and Hites 2005; Cok et al. 2007). Epidemiological studies revealed that perinatal exposure to PCBs exerted a developmental neurotoxicologic effect on humans (Nakai and Satoh 2002; Schantz et al. 2003). In Taiwan, pregnant mothers were accidentally exposed to PCBs through the ingestion of contaminated rice oil and neurodevelopmental abnormalities were observed in their offspring (Chen et al. 1992). Children born to mothers who ingested PCB-contaminated fish also exhibited neurological impairment (Jacobson and Jacobson 1996). This evidence confirmed the long-term impact of PCBs on cognitive deficits and intellectual dysfunction. In experimental studies, the effects of PCBs on learning and memory, and the mechanisms of dysfunctions have been reported (Gilbert and Crofton 1999; Faroon et al. 2001); however, the effect of prenatal exposure to PCB with special reference to the impact on emotionality has not been explored in-depth to date.

3,3′,4,4′,5-pentachlorobiphenyl (PCB126), benzene rings of non-*ortho*-substituted PCB, may assume a planar configuration and have been suggested to be dioxin-like, the most toxic PCB congener (Safe 1990). PCB126 increased adrenocorticotropic hormone in pituitary cells that stimulate the secretion of corticosterone from the adrenal cortex (Bestervelt et al. 1998). PCB126 raised cortisol levels in human adrenocortical cells (Li and Wang 2005). Serum corticosterone was increased by exposure to Aroclor 1254, a PCB mixture which contains PCB126 (Kodavanti et al. 2001), in mice and rats (Sanders et al. 1974; Miller et al. 1993). Thus, PCB126 elevates serum corticosterone/cortisol levels in both direct and indirect manners. Recently, Zagron and Weinstock (2006) revealed that high-level maternal corticosterone was responsible for the anxiogenic properties of offspring. From this evidence, we hypothesized that maternal exposure to PCB126 would cause emotional disturbance in offspring.

In the present study, rats were exposed to PCB126 during pregnancy, and the emotionality of their offspring was evaluated in terms of anxiogenicity. Moreover, the effect of prenatal exposure to PCB126 on the HPA axis was examined by measuring serum corticosterone levels before and after forced swim stress. Prenatal exposure to PCBs possibly exerts motor dysfunction (Chen et al. 1985; Nguon et al. 2005); thus, the effect of prenatal exposure to PCB126 on motor coordination was also examined.

## Materials and Methods

*Subjects*

Twenty 10-week-old female and ten 12-week-old male Sprague-Dawley rats were purchased from SLC, Japan and acclimated for 1 week prior to the start of the experiment. Rats were housed in a cage at 23 ± 2°C, 60 ± 10% humidity, and with lights on daily from 7:00 a.m. to 7:00 p.m. in a controlled room, and received laboratory chow and water *ad libitum*. Two female rats were cohabited overnight with a male rat. Females with sperm in their vaginal smears the next morning were regarded as pregnant and the day was designated as gestation day 0. Our preliminary study revealed that prenatal oral exposure to 100 μg/kg of PCB126 on gestational day 15 reduced neonatal body weight at birth. On the other hand, 30 μg/kg of PCB126 had no effect (6.9 ± 0.1 g [$n$ = 6] vs 7.2 ± 0.3 g [$n$ = 6], normal vs 30 μg/kg of PCB126, $p$ = 0.3778). Thus, ten pregnant rats were treated orally with PCB126 at a dose of 30 μg/kg and the other ten pregnant rats with corn oil, its vehicle, on gestational day 15. All newborns were weighed on postnatal day 1 and each litter was reduced randomly to four males and four females. Only three females were born to one dam that exposed to corn oil although number of male offspring of the dam was nine. In this case, extra one male nursling of the litter was added so that the total number was eight. All female offspring were discarded at weaning. Male offspring were subjected to the present study at 4-5 weeks old. One animal from each litter was used for each behavioral test. Each animal experienced only one behavioral test. Thirty-six male offspring of rats with prenatal exposure to PCB126 and 36 male offspring with prenatal exposure to corn oil were used in the present study. The care and handling of the animals were in accordance with the "Azabu University Animal Experiment Guidelines; April 2000".

*Rotating rod test*

It was examined whether motor coordination of PCB126 rats was impaired using the rotating rod test. Rats were placed on a rotating rod (10 cm diameter,

Natsume, Tokyo) which was rotated at 3 rev./min for 10 min to habituate them to the apparatus. The next day, the rats were again placed on the rotating rod and the speed was increased in the order of 3, 5, 8, 10, and 15 rev./min every 1 min. The number of rats that successfully walked at each speed was recorded.

*Locomotor activity and anxiety-related behavior in an open field*

Locomotor activity was measured in a black wooden box (45 × 45 × 40 cm depth). The apparatus was located under concealed lighting which gave low luminance (55 lx). A rat was placed in the center of the box and left for 15 min. Its behavior was recorded by a digital video camera set above. The number of rearings and duration of grooming behavior, which are indices of anxiety (De Souza Spinosa et al. 1999; Moreira et al. 2000; Sonavane et al. 2002; Kalueff and Tuohimaa 2005), were measured by an observer blinded to the rats' history. The distance moved horizontally and the percent time spent in the 25 cm square in the center were measured using a computer-aided behavior analysis system (SMART, Bio Research Center, Aichi). The apparatus was cleaned using ethanol prior to each experiment.

*Social interaction*

Anxiogenic responses were measured utilizing a modified social interaction test (Sajdyk et al. 2002). A rat with prenatal exposure to either corn oil or PCB126 was put into an arena for the social interaction test (45 × 45 × 40 cm depth) with a novel partner rat of the same sex and of similar weight. Their behavior was video-recorded for 5 min with a digital video camera above. The time spent in social interaction (sniffing, following, grooming the partner, and wrestling) of the test animal provided a measure of anxiety. These measurements were performed by an observer blind to the rats' history.

*Serum corticosterone level under non-stressful conditions and after forced swim stress*

A Plexiglas cylinder (20 × 50 cm, diameter × height) filled with water (25°C) 30 cm in depth was used as a swim stress device. Each rat was put into the cylinder for 15 min and trunk blood was collected in a glass tube by decapitation immediately after forced swim stress. Rats were quickly removed from their home cage and trunk blood was collected as a non-stressful control. All blood collections were performed between 14:00–15:00. Serum corticosterone levels of trunk blood were measured using an RIA kit (Amersham, UK).

*Statistical analyses*

Results are expressed as the means ± S.E.M. or means + S.E.M. The effect of prenatal exposure to PCB126 was analyzed with unpaired *t*-test for body weight, locomotor activity and anxiety-related behavior in an open field, social interaction, and serum corticosterone level, and Fisher's exact probability test for the incidence of the rota-rod test. A $p$ value $< 0.05$ was considered significant.

## Results

### *Body weight*

The body weights of male rats with prenatal exposure to corn oil and PCB126 on postnatal day 1 were 7.0 ± 0.1 g and 7.0 ± 0.1 g, respectively, with no statistical significance between the two groups.

### *Rotating rod test*

The effect of prenatal exposure to PCB126 on motor coordination was examined using a rotating rod. At 3, 5, and 8 rpm, all rats accomplished the walking task (Table 1). At 10 and 15 rpm, 2–4 rats failed to accomplish the walking

TABLE 1. Effect of prenatal exposure to PCB126 (30 μg/kg, PO) or Corn oil on motor coordination in rotating rod test.

| | Revolution (rpm) | | | | |
|---|---|---|---|---|---|
| | 3 | 5 | 8 | 10 | 15 |
| Corn oil ($n = 8$) | 8/8 | 8/8 | 8/8 | 6/8 | 4/8 |
| PCB126 ($n = 8$) | 8/8 | 8/8 | 8/8 | 8/8 | 5/8 |

Number of rats walking on the rotating rod for 1 min/Total number of rats is shown in the table.

task (Table 1); however, there was no difference between the two groups.

*Locomotor activity and anxiety-related behavior in an open field*

Total horizontal distance moved during 15 min in an open field was not affected by prenatal exposure to PCB126 (Fig. 1A). On the other hand, time spent in the center of the open field and the number of rearings fell (Fig. 1B and 1C), and total grooming duration was extended (Fig. 1D).

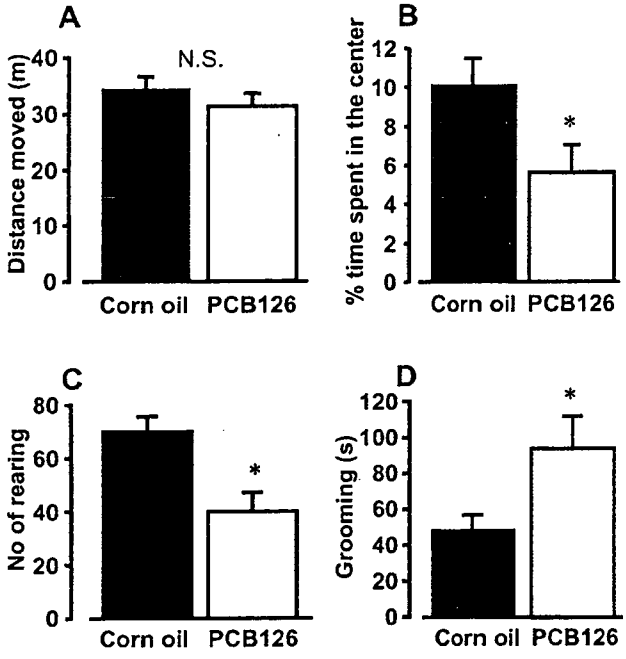


Fig. 1. Effect of prenatal exposure to PCB126 (30 μg/kg, PO) on behavioral parameters. Rats with prenatal exposure to corn oil or PCB126 were put into an open field for 15 min and locomotor activity (A), % time spent in the central area (B), number of rearings (C), and grooming duration (D) were measured. Data are presented as the mean + S.E.M. of 10 animals.
N.S., not significant. $^{*}p < 0.05$ vs corn oil, unpaired *t*-test.

*Social interaction*

Time spent in social interaction during the 5 min test period was 19.9 ± 2.6 s in rats with prenatal exposure to PCB126. This time was significantly shorter than that with prenatal exposure to corn oil, 32.5 ± 2.9 s (Fig. 2)

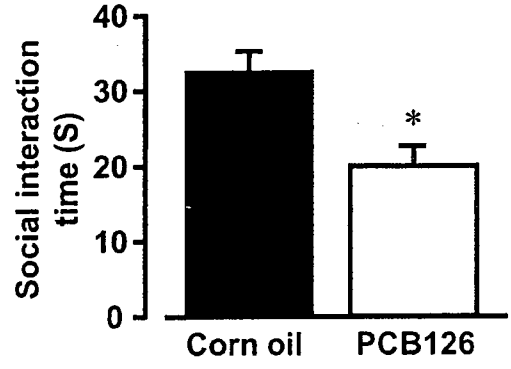


Fig. 2. Effect of prenatal exposure to PCB126 (30 μg/kg, PO) on social interaction. Rats with prenatal exposure to corn oil or PCB126 were put together with a novel partner rat and the time spent in social interaction (sniffing, following, grooming the partner, and wrestling) was measured. Data are presented as the mean + S.E.M. of 9 animals.
$^{*}p < 0.05$ vs corn oil, unpaired *t*-test.

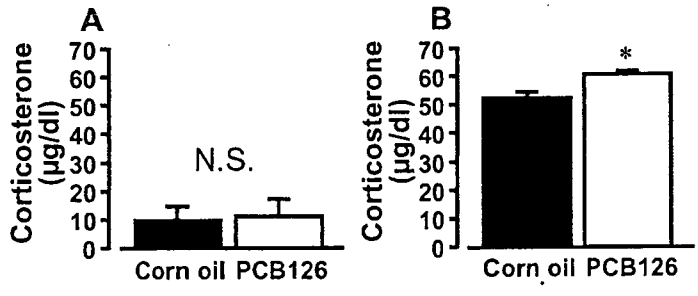


Fig. 3. Serum corticosterone level under non-stressful conditions (A) and after forced swimming (B) in rats with prenatal exposure to corn oil or PCB126. Data are presented as the mean + S.E.M. of 4 (A) and 5 (B) animals.
N.S., not significant. $^{*}p < 0.05$ vs corn oil, unpaired *t*-test.

*Serum corticosterone level under non-stressful conditions and after forced swim stress*

The serum corticosterone levels under non-stressful conditions were not affected by prenatal exposure to PCB126 (Fig. 3A); however, the increase in the serum corticosterone level induced by forced swim stress was significant (Fig. 3B).

## Discussion

The present study revealed that open field performance was greatly influenced by prenatal exposure to PCB126. In rodents, grooming is particularly sensitive to stress and exogenous manipulation, and generally facilitated under stressful situations (Moyaho and Valencia 2002). In contrast, rearing, an exploratory behavior, is decreased under anxiogenic conditions as anxiogenic agents exerted a decrease in the number of rearings in an open field (De Souza Spinosa et al. 1999; Sonavane et al. 2002). Together with the evidence of decreased time spent in the center of the open field and decreased social interaction, which is a representative index of anxiety (Sajdyk et al. 2002), it is suggested that rats with prenatal exposure to PCB126 are sensitive to stress and have a propensity to develop anxiety.

Maternal stress exerted emotional disturbance, including anxiogenic behavior, through modification of the feedback mechanism of the offspring HPA (Henry et al. 1994; Patin et al. 2005). As the anxiogenic behavior of prenatally-stressed rats was abolished by maternal adrenalectomy, excess maternal corticosterone may cause this emotional disturbance (Zagron and Weinstock 2006). Literature data show that PCB has a property to elevate serum corticosterone when administered orally (De Krey et al. 1993; Miller et al. 1993), and the data of the present study show that prenatal exposure to PCB causes a disturbance of HPA response. It is conceivable from this evidence that the underlying mechanisms of the emotional disturbance induced by prenatal stress and PCB exposure may be common to both. Thus, dysfunction of the HPA axis may, at least in part, contribute to the emotional dysfunction of rats with prenatal exposure to PCB126. As hippocampal corticoid receptors decreased in prenatally-stressed rats (Henry et al. 1994), this mechanism may be involved in the dysfunction of HPA induced by prenatal exposure to PCB126. Further studies are necessary to prove this hypothesis.

To stay on the rotating rod, the rats required complex motor skills, including motor coordination and precise postural control. Nevertheless, prenatal PCB126 did not affect their performance, even at the highest rotation speed. Together with evidence that locomotor activity in an open field was not different in the two groups, prenatal PCB126 at the dose examined may not exert motor dysfunction, at least at the age subjected to the experiment. In previous studies, however, maternal exposure to PCB elicited motor dysfunction in the rotating rod test (Nguon et al. 2005) and swimming test (Pantaleoni et al. 1988). The former and latter studies administered Aroclor 1254 and Fenclor 42, respectively, both of which are commercial PCB mixtures. The dosing period was gestation day 11 through postnatal day 21, and gestation day 6 through 15, respectively. We administered PCB126 once on gestation day 15. The different types of PCB and dosing period may have caused the different effects on motor function.

The concentration of PCB126 in adipose tissue of Japanese was 120–730 pg/g (Kannan et al. 1989). When PCB126 was administered orally to rats at a daily dose of 10 $\mu$g/kg for 13 weeks, the concentration in adipose tissue was 645 ng/g (Van Birgelen et al. 1994). Thus, the amount of PCB126 in the adipose tissue of the present study is estimated to be about 1,000 times higher than that in humans. As the toxic effect is dependent on the dose, it is doubtful that PCB affects the central nervous system in humans. Indeed, there is little evidence available about the anxiogenic effect of prenatal exposure to PCB in humans; however, etiological study revealed that chronic inhalation of low chlorinated PCBs in school buildings was associated with increased emotional complaints in humans (Peper et al. 2005); therefore, we cannot reject the possibile effect of prenatal exposure to PCB126 on emotionality. Besides the toxicity of PCBs, additive or synergic neurodevelopmental effects of prenatal exposure

to PCBs and MeHg, which is also an environmental contaminant, have been evidenced in terms of motor dysfunction (Roegge and Schantz 2006) and toxicity to pituitary cells (Johansson et al. 2006). A cohort study of child development from the effects of perinatal methylmercury and environmental pollutants (Nakai et al. 2004) may uncover the intrinsic effect of prenatal exposure to PCB.

In summary, prenatal exposure to PCB126 decreased rearing and time spent in a central area, increased grooming in an open field, and decreased social interaction, although it did not affect total locomotor activity. This evidence suggests that rats with prenatal exposure to PCB126 are vulnerable to stress. As the serum corticosterone level increased in stressed rats with prenatal PCB126, dysfunction of the HPA axis may be one of the causes of anxiogenic properties.

## Acknowledgments

The authors thank Drs. Kinji Shirota and Fumiaki Akahori for encouragement. This work was supported in part by the "High-Tech Research Center" Project for Private Universities: matching fund subsidy from the Ministry for Education, Culture, Sports, Science and Technology of Japan, 2002 –2006.

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Cell Tissue Res
DOI 10.1007/s00441-007-0546-8

REVIEW

# Stem cells and neonatal brain injury

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Received: 22 August 2007 / Accepted: 25 October 2007


**Abstract** Recent advances in regenerative medicine and in our understanding of neurogenesis may lead to new ways of recovering neuronal function lost or damaged during the perinatal period; such injuries are not amenable to conventional therapies. We review recent experimental studies based on immature rodents models of neonatal brain injury, especially hypoxic-ischemic encephalopathy. The developing brain is revealed to have considerable potential with respect to proliferation and migration to the injured site. However, the generation of fully differentiated neurons is extremely limited after brain injuries. Aggressive efforts to adjust the environment of the damaged brain in which tissue regeneration is occurring or more cautious stem cell transplantation will be required for the successful treatment of developmental brain injury.

**Keywords** Neonatal brain injury · Hypoxia-ischemia · Neural stem/progenitor cell

## Introduction

Neonatal brain injury exhibits some unique aspects that are not seen in adult brain damage. The abnormal symptoms and signs characterizing neonatal brain injury are thought to be mainly associated with adverse events that happen in the antenatal period (Badawi et al. 1998a, 1998b). Amongst the precipitating events are trauma, metabolic abnormalities, infection, hypoxia, ischemia, and the presence of toxins (Volpe 2000). Another factor is that antenatal brain injuries are rarely diagnosed in the neonatal period but instead are discovered later in life and are diagnosed as, for example, cerebral palsy, mental retardation, epilepsy, cognitive disturbances, and learning disability (van de Riet 1999). Relatively minor brain injuries received during the perinatal period can also evolve into variable functional impairments as infants grow (Mishima et al. 2005).

Clearly, therapeutic modalities are not easy to perform just after an insult occurs during the perinatal period. This is especially true in the fetal brain. With regard to hypoxic-ischemic encephalopathy, which accounts for about 10% of neonatal brain injuries (Hankins and Speer 2003), the intensive monitoring of intrapartum fetal well-being together with fetal heart rate patterns (Parer and Ikeda 2007), the application of an adequate neonatal resuscitation program, and hypothermic treatment for moderately affected infants (Gluckman et al. 2006), all seem to have a beneficial impact on the outcome of hypoxic-ischemic-induced brain injury. However, to date, no definitive evidence has been provided on the possible decrease in cerebral palsy or other developmental brain disorders.

Once brain damage is established, the resulting chronically injured status dose not seem to be amenable to conventional therapies. Thus, regenerative therapies, cell transplantation, and gene therapy have been proposed as possible methods to overcome long-lasting devastating disorders. Recent advances in regenerative medicine and in our understanding of neurogenesis may lead to ways of recovering neuronal functions that may be lost in perinatal

This work was supported by a Research Grant for Cardiovascular Diseases (18C-1) from the Ministry of Health, Labour and Welfare.

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brain injury. In this paper, we describe recent experimental results, including data from our laboratory, regarding neuronal stem cell and neonatal brain damage. We have limited this review to hypoxic-ischemic brain injury as a model of disorder in neonatal brain injury, because it has been most investigated and is the best described (Park et al. 2002a).

## Endogenous stem cell proliferation in response to hypoxic-ischemic injury

The immature and developing brain might be expected to possess a more potent capacity with respect to neurogenesis and plasticity compared with the adult brain. Neurogenesis comprises cell proliferation, migration, and differentiation (Iwai et al. 2002, 2003). We have carried out a series of experiments to elucidate endogenous behavior, in terms of these factors, in the immature rat brain in response to a unilateral hypoxic-ischemic insult (Hayashi et al. 2005; Ikeda et al. 2005; Iwai et al. 2006). For these studies, we used the Rice-Vannucci rat model in which a 2-h period of hypoxia was imposed after ligation of the left carotid artery. This choice of model was based on its wide use in neonatal hypoxic-ischemic encephalopathy (Vannucci 1990). The contralateral (non-ligated) brain hemisphere acts as a control for hypoxic stress, in addition to non-hypoxic-ischemic sham controls. The stable survival of the treated rats is also an advantage in this model, because long-term effects can be readily evaluated (Hagberg et al. 2002).

In order to assess the proliferation of neuronal stem cells, bromodeoxyuridine (BrdU), which is incorporated into DNA as a nucleotide base, was used to label the dividing cells. We performed an intraperitoneal injection of single-dose BrdU (50 mg/kg) on postnatal day 7 (P7) in the basal group and on 1 day (P8), 7 days (P14), 14 days (P21), and 21 days (P28) after hypoxia-ischemia. The brain was retrieved 2 h after BrdU injection. When immunoreactive BrdU-positive cells were counted in the subventricular zone (SVZ) of the lateral ventricle (the most important germinal matrix of the rat), the number of BrdU-labeled cells in the injured (left) side of the hypoxic-ischemic brain was twice the level of that in the sham controls at 7 days after hypoxia-ischemia. Interestingly, the non-injured (right) side of the hypoxic-ischemic brain showed a comparable number of BrdU-positive cells to the injured side (Fig. 1). Both sides returned to the control level by 21 days after hypoxia-ischemia.

The finding that a unilateral hypoxic-ischemic insult enhanced neural stem cell proliferation, not only in the injured brain, but also on the other uninjured side indicates that humoral factors might influence proliferation both. This supposition is supported by recent experiments (Park et al. 2006a) in which a soluble fraction and a membrane fraction have been extracted from hypoxic-ischemic neonate brain hemispheres, in acute and chronic stages; the extracts significantly facilitate mitosis of a neuronal stem cell line in vitro.

Our results are consistent with previous reports of increased proliferation and neurogenesis in the SVZ region after hypoxic-ischemic stress in neonatal rodents (Ong et al. 2005; Park et al. 2006a; Plane et al. 2004; Qiu et al. 2007; Yang and Levison 2006). Although increased proliferation has been seen in both SVZs (injured/non-injured), the phenotype of the proliferating cells appears to be significantly different. Levison and colleagues (Felling et al. 2006; Yang and Levison 2006) have harvested SVZ from both sides of neonatal rats at 48 h of recovery and have cultured the cells in vitro as neurospheres in the presence of the fibroblast growth factor (FGF-2) and epidermal growth factor (EGF). When the neurospheres are further differentiated in growth-factor-free medium for 5 days, injured-side-derived neurospheres are significantly larger than those from the non-injured side or from a sham-control and have more cells showing tripotential neural stem/progenitor cell markers, such as nestin, Notch1, and EGF-receptor. Injured-side-derived neurospheres also differentiate preferentially into oligodendrocytes and neuronal cells than into astroglia. These findings indicate that, in the injured side, increased proliferation within the neuronal stem/progenitor cell lineage occurs 2 days after hypoxia-ischemia. We speculate that this effect compensates for the vulnerability in neurons and oligodendrocytes in the developmental stage (Back et al. 2001, 2002).

## Endogenous stem cell migration following hypoxic-ischemic injury

Doublecortine (DCX) is a marker for migrating neuronal precursor cells, the early stage of neuronal differentiation from multipotent neuronal stem cells (Francis et al. 1999; Gleeson et al. 1999). We have used an anti-DCX antibody to evaluate the migrating activity of the precursor. Rat brains were retrieved 7 (P14), 14 (P21), and 21 (P28) days after hypoxic-ischemic insult on postnatal day 7. Each rat was administered BrdU (50 mg/kg, six times, 12 h apart) for the last 3 days before brain retrieval. At 7 days after hypoxia-ischemia, 456±72 BrdU-positive cells/mm$^2$ were found around the cortical infarcted area, with 24% of BrdU-positive cells being DCX positive. By contrast, there were only 88 and 106 BrdU-positive cells in the corresponding areas of uninjured cortex and sham-control cortex, respectively. Moreover, in control cortices, few cells (only 0.6% of the BrdU-positive cells each) were positive for DCX. The total number of BrdU-positive cells in the infarcted cortex decreased gradually 14 and 21 days after hypoxia-

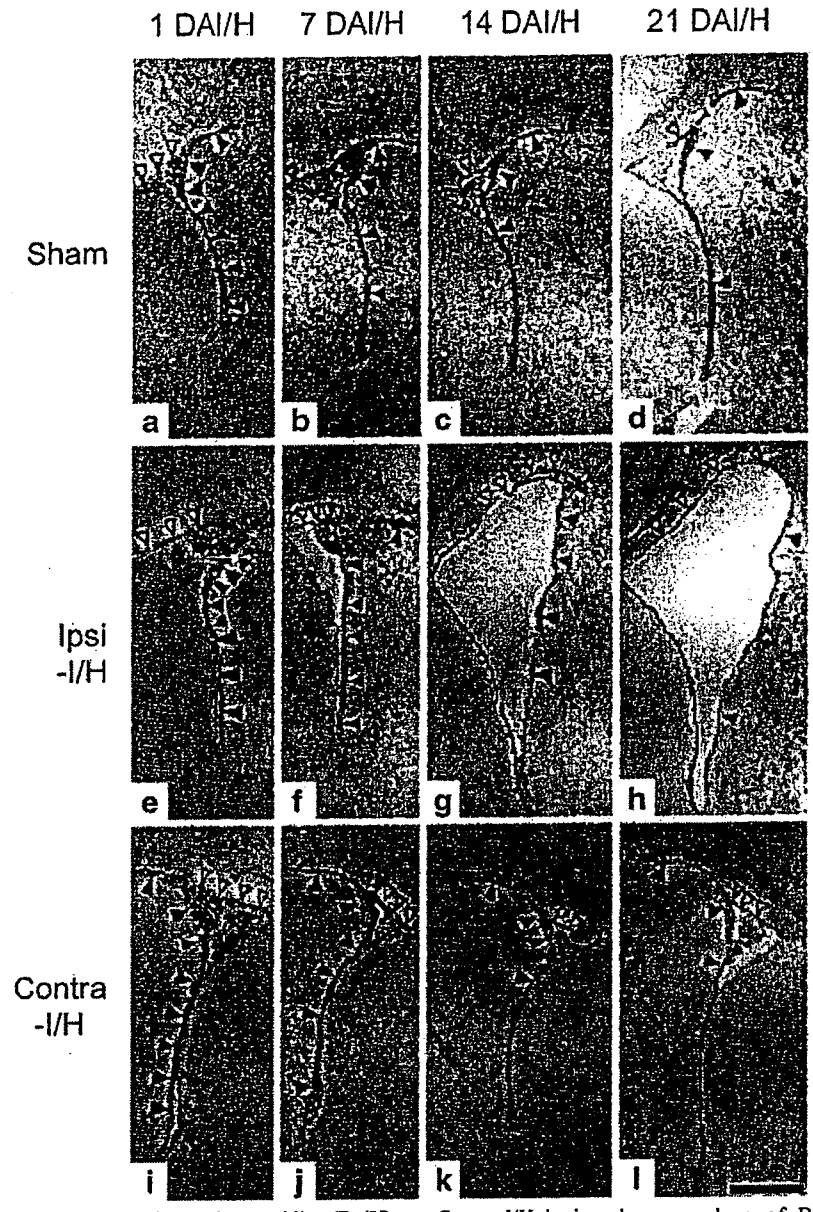


**Fig. 1** Immunohistochemical staining for bromodeoxyuridine (BrdU) in the subventricular zone (SVZ) of sham-control animals (*Sham*, a–d), the ipsilateral side of ischemia/hypoxia brain (*Ipsi-I/H*, e–h), and the contralateral side of ischemia/hypoxia brain (*Contra-I/H*, i–l), 1 day (*1 DAI/H*, a, e, i), 7 days (*7 DAI/H*, b, f, j), 14 days (*14 DAI/H*, c, g, k), and 21 days (*21 DAI/H*, d, h, l) after insult. In SVZa, which lines the lateral wall of the lateral ventricle (LV), in both *Ipsi-I/H* and *Contra-I/H* brains, large numbers of BrdU-labeled cells (*black arrowheads*) were detected from 1 to 7 days after insult compared with the sham control. Note that, in SVZb, which lines the upper wall of the LV, in both *Ipsi-I/H* and *Contra-I/H* brains, large numbers of BrdU-labeled cells (*open arrowheads*) could be detected from 7 to 21 days after insult compared with the sham control. Data were derived from Iwai et al. (2006)

ischemia, whereas the percentage of DCX-positive cells remained constant at around 25%. This finding of a significant migration of DCX-positive newly divided cells around the infarction area, but not on the non-injured side, is consistent with other experiments on neonatal rats or mice (Felling et al. 2006; Yang and Levison 2006). The rate of migration in the neonatal brain seems to be greater than that in the adult brain (Jin et al. 2001; Zhang et al. 2001), indicating the powerful regenerative potential of the neonatal brain.

Several candidate chemotaxic factors have been proposed to attract neuronal progenitor cells. Quantitative polymerase chain reaction of the isolated brain from neonatal rats allowed to recover for 3 and 14 days after hypoxia-ischemic treatment showed that monocyte chemoattractant protein-1 (MCP-1) significantly increased in the

cortex, and that its receptor, CCR-2, also increased in the SVZ and cortex (Yang et al. 2007). Stromal derived factor-1 (SDF-1) and its receptor CXCR-4 are other potential candidate chemotaxic factors (Miller et al. 2005).

One important question is whether a neural stem cell proliferating in the SVZ of the non-injured side migrates into the injured side to take part in regeneration. In recent experiments, Park et al. (2006a) clearly demonstrated that this can occur through the corpus collosum and fimbria. In this study, they injected the clonal multipotent neural precursor cell line (C17.2) derived from the external germinal layer of the neonatal mouse cerebellum and incorporating the lacZ-expressing molecule as a reporter marker into neonatal rat brain; the engrafted C17.2 cells migrated from the non-injured hemisphere to the injured side.

## Endogenous stem cell differentiation after hypoxic-ischemic injury

Previously, we have used an anti-neuronal nuclear antigen (NeuN) antibody to detect matured neurons, together with BrdU, as an early marker of cell proliferation (Ikeda et al. 2005). BrdU was administrated at days 5–7 (50 mg/kg intraperitoneally, six times, in total) after hypoxia-ischemia on postnatal day 7, and rat brains were extracted 14 (P21), 28 (P35), and 42 days (P49) later. At 14 days after hypoxia-ischemia, 121 BrdU-positive cells/mm$^2$ were found around the injured cavity. Unexpectedly, and rather disappointingly, only 1% of BrdU-positive cells were mature neurons (NeuN-immunopositive; Fig. 2). BrdU-positive cells represented cells that would have divided 7–9 days previously (i.e., 5–7 days after the hypoxic-ischemic insult), when the cells would have been undergoing maximum proliferation in the SVZ. This cohort of cells must have migrated to the infarcted area in the cortex at 7 days after hypoxia-ischemia.

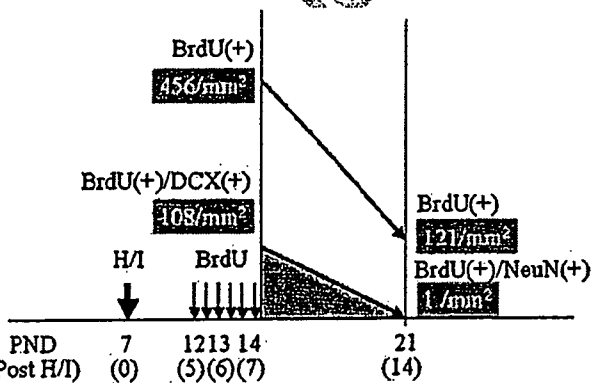


Fig. 2 Schematic explanation of the limited differentiation from neural progenitor cells that are doubly immunopositive for BrdU and doublecortine (*DCX*) to mature neurons positive for BrdU and neuronal nuclear antigen (*NeuN*). At 7 days after hypoxic-ischemic (*H/I*) treatment, 108 of 456 BrdU-positive cells that were around the infarcted area were immunopositive for DCX. At 14 days after H/I treatment, however, only a single cell differentiated into a mature neuron out of 108 migrating neural progenitor cells (*PND* postnatal day). Data derived from Ikeda et al. (Ikeda et al. 2005)

Few newly formed neurons could be found no more than 28 days after the hypoxic-ischemic insult. This finding is consistent with the observation by Morshead and van der Kooy (1992) that most newly generated neurons in the adult appear to undergo programmed cell death, rather than surviving to make a neuronal network. Furthermore, the nuclear morphology of the cells doubly immunopositive for BrdU and NeuN suggests that they were "interneurons", rather than pyramidal neurons, as observed by Yang et al. (2007). These newly recruited neurons are apparently ineligible to connect with other neurons to make a neural network or to contribute, even partially, to functional recovery. This significantly impaired ability to differentiate contrasts with the successful differentiation into neurons and astroglia of externally injected human neural stem cells, in experiments with normally developing animal brains (Ourednik et al. 2001). Our data may be explained by the poor environment provided by the infarcted cortex. There may be poor neural inputs and outputs or a poor humoral milieu (such as low levels of neurotrophic factors). The reduced self-repairing ability in this model seems to be related to the large area of cell degeneration. Daval et al. (2004) have reported complete self-repair in the hippocampus region CA1 of neonatal rats. In their study, transient hypoxia results in merely a small reduction in the neuronal cells in this region. Thus, the hypoxic-ischemic insult in the present study may be too severe for successful neuronal self-repair.

The finding of the severely limited ability to differentiate from neuronal precursor cells to mature neurons implies that attempts to produce functional recovery by simple stem cell transplantation is unlikely to an effective method of treating neonatal hypoxic-ischemic encephalopathy.

## Neuroregeneration in neonatal hippocampus

Fewer studies of the neuroregeneration of other areas have been reported compared with those of the neonatal brain cortex. The complete self-repair of damage in the CA1 region of the hippocampus in the study of Daval et al. (2004; see also above) may be alternatively explained by the regional difference of the neonatal brain. Recently, Qiu et al. (2007) have studied endogenous proliferation and differentiation after hypoxia-ischemia in neonate (postnatal day 9) and juvenile (postnatal day 21) mouse. Contrary to what has been generally assumed, their results indicate that the juvenile brain has a greater capacity for neurogenesis