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## 1 Do-Calculus: A Tutorial on Causal Reasoning with Graphs

This tutorial introduces **do-calculus**, Pearl's formal system for reasoning about causal effects using directed acyclic graphs (DAGs). Do-calculus provides a set of graph-based rules that let us transform interventional queries like  $p(y | do(x))$  into expressions we can estimate from observational data.

## 1.1 What You'll Learn

- The difference between observational conditioning and causal intervention
- How to represent causal assumptions using DAGs
- The concept of d-separation and how it determines conditional independence
- The three rules of do-calculus
- How to derive the back-door and front-door adjustment formulas
- Common pitfalls like collider bias

## 1.2 Prerequisites

- Basic probability theory (conditional probability, Bayes' rule)
- Familiarity with the potential outcomes framework (helpful but not required)

## 1.3 Table of Contents

1. Basic Objects and Notation
  2. The Purpose of Do-Calculus
  3. Graph Surgery: The Do-Operator
  4. D-Separation: The Engine of Causal Reasoning
  5. The Three Rules of Do-Calculus
  6. Worked Example: Back-Door Adjustment
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  8. Worked Example: The Collider Trap
  9. Applications in Computational Biology
  10. Practical Checklist
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## 1.4 1. Basic Objects and Notation

### 1.4.1 Variables

In causal inference, we typically work with:

- $X$ : **Treatment/intervention variable** (drug exposure, gene knockdown, CRISPR perturbation)
- $Y$ : **Outcome** (phenotype, gene expression, survival)
- $Z, W$ : **Covariates** (cell state, batch, donor, disease severity)
- $U$ : **Unobserved confounder** (latent factors, unmeasured biology)

### 1.4.2 Two Kinds of Probability Statements

**Observational (seeing):**

$$p(y | x) \quad \text{or} \quad p(y | x, z)$$

This is standard conditional probability: “Among samples where  $X = x$ , what is the distribution of  $Y$ ? ”

**Interventional (doing):**

$$p(y | do(x))$$

This means: “What would  $Y$  look like if we *forced*  $X$  to take value  $x$ , regardless of what would naturally cause  $X$  to be that value? ”

### 1.4.3 The Critical Distinction

In general:

$$p(y | do(x)) \neq p(y | x)$$

**Why?** Conditioning doesn't break confounding, but interventions do.

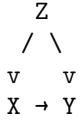
**Example:** Consider the relationship between carrying a lighter and lung cancer. Observationally,  $p(\text{cancer} | \text{lighter})$  is elevated because smokers carry lighters. But  $p(\text{cancer} | do(\text{lighter}))$ —the effect of *forcing* someone to carry a lighter—is essentially zero. The intervention breaks the confounding path through smoking.

### 1.4.4 Graph Language: Directed Acyclic Graphs (DAGs)

We represent causal assumptions with a **directed acyclic graph (DAG)**:

- **Nodes** represent variables
- **Directed edges** ( $\rightarrow$ ) represent direct causal influence
- **Bidirected edges** ( $\leftrightarrow$ ) represent unobserved common causes (shorthand for a hidden  $U$  causing both endpoints)
- **Acyclic** means no variable can cause itself through any path

**Example DAG:**



Here  $Z$  is a **confounder**: it causes both  $X$  and  $Y$ , creating a spurious association between them.

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## 1.5 2. The Purpose of Do-Calculus

Do-calculus addresses two fundamental tasks:

1. **Identification:** Can we rewrite  $p(y | do(x))$  using only observational quantities like  $p(y | x, z), p(z | x)$ , etc.?
2. **Estimation:** Once identified, estimate the causal effect from data.

Do-calculus solves task (1) using graph-based rules. If you cannot rewrite the interventional query in observational terms, then your assumptions and measurements are insufficient to identify the causal effect from observational data alone—you need experiments.

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## 1.6 3. Graph Surgery: The Do-Operator

The  $do(x)$  operator has a precise graphical interpretation: **cut all arrows into  $X$ , then set  $X = x$ .**

### 1.6.1 Notation

- $G$ : the original causal graph
- $G_{\bar{X}}$ : the **mutilated graph** with all incoming edges to  $X$  removed (“bar  $X$ ”)
- $G_{\underline{X}}$ : the graph with all outgoing edges from  $X$  removed (“underline  $X$ ”)

## 1.6.2 Intuition

When we intervene on  $X$ : \* We sever  $X$  from its natural causes \*  $X$  becomes like a randomized treatment—its value is set externally \* All downstream effects of  $X$  remain intact

**Example:**

Original graph:

$$Z \rightarrow X \rightarrow Y$$

After  $do(x)$  (graph  $G_{\bar{X}}$ ):

$$Z \quad X \rightarrow Y$$

The arrow  $Z \rightarrow X$  is cut. Now  $X$  is independent of  $Z$  in the mutilated graph.

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## 1.7 4. D-Separation: The Engine of Causal Reasoning

**D-separation** (directional separation) is the key concept that determines which conditional independences hold in a DAG. It's the foundation for all do-calculus rules.

### 1.7.1 Definition

A set of variables  $S$  **d-separates**  $A$  from  $B$  in a DAG  $G$  if  $S$  blocks every path between  $A$  and  $B$ .

### 1.7.2 The Three Path Types

To understand d-separation, we need to understand three types of path structures:

#### 1.7.2.1 1. Chain (Mediation): $A \rightarrow M \rightarrow B$

$$A \rightarrow M \rightarrow B$$

- **Unconditionally:**  $A$  and  $B$  are associated (information flows through  $M$ )
- **Conditioning on  $M$ :** Blocks the path;  $A \perp\!\!\!\perp B | M$

**Example:** Smoking  $\rightarrow$  Tar in lungs  $\rightarrow$  Cancer. Conditioning on tar blocks the association between smoking and cancer through this path.

#### 1.7.2.2 2. Fork (Confounding): $A \leftarrow C \rightarrow B$

$$A \leftarrow C \rightarrow B$$

- **Unconditionally:**  $A$  and  $B$  are associated (both caused by  $C$ )
- **Conditioning on  $C$ :** Blocks the path;  $A \perp\!\!\!\perp B | C$

**Example:** Genetics  $\rightarrow$  Smoking, Genetics  $\rightarrow$  Cancer. Conditioning on genetics blocks the spurious association.

#### 1.7.2.3 3. Collider (Inverted Fork): $A \rightarrow C \leftarrow B$

$$A \rightarrow C \leftarrow B$$

- **Unconditionally:** Path is **blocked**;  $A \perp\!\!\!\perp B$
- **Conditioning on  $C$ :** **Opens** the path;  $A$  and  $B$  become associated!

**Example:** Talent  $\rightarrow$  Hollywood success  $\leftarrow$  Beauty. Among successful actors (conditioning on the collider), talent and beauty become negatively correlated—the “explain-away” effect.

### 1.7.3 D-Separation Algorithm

To check if  $S$  d-separates  $A$  from  $B$ :

1. List all paths between  $A$  and  $B$
2. For each path, check if it's blocked by  $S$ :
  - A chain or fork is blocked if the middle node is in  $S$
  - A collider is blocked if the collider (and all its descendants) is NOT in  $S$
3. If ALL paths are blocked,  $A$  and  $B$  are d-separated given  $S$

### 1.7.4 Worked D-Separation Examples

#### Example 1: Simple Confounding



- Path  $X \leftarrow Z \rightarrow Y$  is a fork at  $Z$
- $X \perp\!\!\!\perp Y \mid Z?$  **Yes** (conditioning on  $Z$  blocks the fork)
- $X \perp\!\!\!\perp Y?$  **No** (path is open)

#### Example 2: Mediation

$$X \rightarrow M \rightarrow Y$$

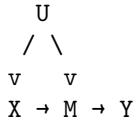
- Path  $X \rightarrow M \rightarrow Y$  is a chain through  $M$
- $X \perp\!\!\!\perp Y \mid M?$  **Yes** (conditioning blocks the chain)
- $X \perp\!\!\!\perp Y?$  **No** (path is open)

#### Example 3: Collider

$$X \rightarrow C \leftarrow Y$$

- Path  $X \rightarrow C \leftarrow Y$  is a collider at  $C$
- $X \perp\!\!\!\perp Y?$  **Yes** (collider blocks the path)
- $X \perp\!\!\!\perp Y \mid C?$  **No** (conditioning opens the collider!)

#### Example 4: Complex Graph



Paths from  $X$  to  $Y$ : 1.  $X \rightarrow M \rightarrow Y$  (chain) 2.  $X \leftarrow U \rightarrow Y$  (fork)

- $X \perp\!\!\!\perp Y?$  **No** (both paths open)
- $X \perp\!\!\!\perp Y \mid U?$  **No** (path 1 still open)
- $X \perp\!\!\!\perp Y \mid M?$  **No** (path 2 still open)
- $X \perp\!\!\!\perp Y \mid M, U?$  **Yes** (both paths blocked)

## 1.8 5. The Three Rules of Do-Calculus

Do-calculus provides three rules for manipulating interventional expressions. Each rule has a graphical condition based on d-separation in modified graphs.

### 1.8.1 Rule 1: Insertion/Deletion of Observations

$$p(y \mid do(x), z, w) = p(y \mid do(x), w)$$

**Condition:**  $Y \perp\!\!\!\perp Z \mid X, W$  in  $G_{\overline{X}}$  (the graph with incoming edges to  $X$  removed)

**Intuition:** After intervening on  $X$ , observing  $Z$  provides no additional information about  $Y$  (given  $W$ ).

### 1.8.2 Rule 2: Action/Observation Exchange

$$p(y \mid do(x), do(z), w) = p(y \mid do(x), z, w)$$

**Condition:**  $Y \perp\!\!\!\perp Z \mid X, W$  in  $G_{\overline{X}, \underline{Z}}$  (incoming edges to  $X$  removed, outgoing edges from  $Z$  removed)

**Intuition:** Under certain graphical conditions, “setting  $Z$ ” is equivalent to “conditioning on  $Z$ ” for predicting  $Y$ .

### 1.8.3 Rule 3: Insertion/Deletion of Actions

$$p(y \mid do(x), do(z), w) = p(y \mid do(x), w)$$

**Condition:**  $Y \perp\!\!\!\perp Z \mid X, W$  in  $G_{\overline{X}, \overline{Z(W)}}$  where  $Z(W)$  denotes  $Z$ -nodes that are not ancestors of any  $W$ -node in  $G_{\overline{X}}$

**Intuition:** Intervening on  $Z$  doesn’t affect  $Y$  once we’ve intervened on  $X$  and conditioned on  $W$ .

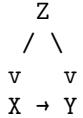
### 1.8.4 Using the Rules

These rules may seem abstract, but they become concrete in worked examples. The key insight: **d-separation in modified graphs determines when you can simplify interventional expressions.**

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## 1.9 6. Worked Example: Back-Door Adjustment

### 1.9.1 The Graph



$Z$  is a confounder: it causes both  $X$  (treatment) and  $Y$  (outcome).

**Goal:** Identify  $p(y \mid do(x))$ .

### 1.9.2 Derivation

#### Step 1: Law of Total Probability

$$p(y \mid do(x)) = \sum_z p(y, z \mid do(x))$$

#### Step 2: Factor the Joint

$$p(y, z \mid do(x)) = p(y \mid z, do(x)) \cdot p(z \mid do(x))$$

So:

$$p(y | do(x)) = \sum_z p(y | z, do(x)) \cdot p(z | do(x))$$

**Step 3: Simplify  $p(z | do(x))$**

In the mutilated graph  $G_{\bar{X}}$ , the edge  $Z \rightarrow X$  is cut. Since  $Z$  has no path from  $X$  in  $G_{\bar{X}}$ :

$$p(z | do(x)) = p(z)$$

**Step 4: Simplify  $p(y | z, do(x))$**

In  $G_{\bar{X}}$ , conditioning on  $Z$  blocks the back-door path. By Rule 2 (action/observation exchange):

$$p(y | z, do(x)) = p(y | x, z)$$

**Step 5: Combine**

$$p(y | do(x)) = \sum_z p(y | x, z) \cdot p(z)$$

This is the **back-door adjustment formula**. It expresses the causal effect entirely in terms of observational quantities.

### 1.9.3 Back-Door Criterion (General)

A set  $Z$  satisfies the **back-door criterion** relative to  $(X, Y)$  if:

1. No node in  $Z$  is a descendant of  $X$
2.  $Z$  blocks every path between  $X$  and  $Y$  that contains an arrow into  $X$

If  $Z$  satisfies the back-door criterion:

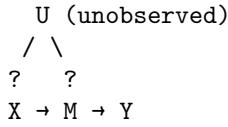
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$$p(y | do(x)) = \sum_z p(y | x, z) \cdot p(z)$$

## 1.10 7. Worked Example: Front-Door Adjustment

The front-door adjustment is remarkable: it allows causal identification **even with unmeasured confounding** between treatment and outcome.

### 1.10.1 The Graph



Where: \*  $U$  is an unobserved confounder between  $X$  and  $Y$  (shown as  $X \leftrightarrow Y$ ) \*  $M$  is a mediator:  $X \rightarrow M \rightarrow Y$  \* All causal effect of  $X$  on  $Y$  goes through  $M$  \* No confounding between  $X$  and  $M$

**Goal:** Identify  $p(y | do(x))$  despite the unmeasured confounder.

### 1.10.2 Derivation

#### Step 1: Marginalize over the Mediator

$$p(y | do(x)) = \sum_m p(y | m, do(x)) \cdot p(m | do(x))$$

#### Step 2: Simplify $p(m | do(x))$

Since  $M$  is caused by  $X$  and there's no confounding between them:

$$p(m | do(x)) = p(m | x)$$

#### Step 3: Simplify $p(y | m, do(x))$

Once we condition on  $M$ ,  $X$  has no direct effect on  $Y$  (all effect goes through  $M$ ). Under the front-door conditions:

$$p(y | m, do(x)) = p(y | do(m))$$

So:

$$p(y | do(x)) = \sum_m p(y | do(m)) \cdot p(m | x)$$

#### Step 4: Identify $p(y | do(m))$ via Back-Door

For the effect of  $M$  on  $Y$ ,  $X$  serves as a valid back-door adjustment set:

$$p(y | do(m)) = \sum_{x'} p(y | m, x') \cdot p(x')$$

#### Step 5: Combine

$$p(y | do(x)) = \sum_m p(m | x) \sum_{x'} p(y | m, x') \cdot p(x')$$

This is the **front-door adjustment formula**—one of the most elegant results in causal inference.

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## 1.11 8. Worked Example: The Collider Trap

This example illustrates why “just control for more variables” is dangerous in causal inference.

### 1.11.1 The Graph

$$X \rightarrow C \leftarrow U \rightarrow Y$$

Where: \*  $C$  is a **collider**: both  $X$  and  $U$  cause  $C$  \*  $U$  is unobserved and causes  $Y$  \* There is no direct effect of  $X$  on  $Y$

### 1.11.2 Analysis

**Without conditioning on  $C$ :** \* Path  $X \rightarrow C \leftarrow U \rightarrow Y$  is blocked at collider  $C$  \*  $X \perp\!\!\!\perp Y$  (no causal effect, no spurious association) \*  $p(y | x) = p(y)$

**With conditioning on  $C$ :** \* Conditioning on collider  $C$  **opens** the path \*  $X$  and  $U$  become associated (given  $C$ ) \* This creates spurious association between  $X$  and  $Y$  \*  $p(y | x, c) \neq p(y)$

### 1.11.3 The Lesson

Conditioning on a collider (or its descendants) can **create** bias where none existed. This is called **collider bias** or **selection bias**.

**Real-world example:** Suppose  $C$  = “admitted to hospital”,  $X$  = “has flu”,  $U$  = “has heart disease”,  $Y$  = “mortality”. Among hospitalized patients (conditioning on  $C$ ), flu and heart disease become negatively correlated (explaining away), which can create spurious associations with mortality.

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## 1.12 9. Applications in Computational Biology

In computational biology, common causal structures include:

### 1.12.1 Confounders

- **Cell state** confounds perturbation effects (cycling cells transfect better AND express genes differently)
- **Batch/donor effects** confound treatment-outcome relationships
- **Disease severity** confounds treatment assignment and outcomes

### 1.12.2 Colliders (Selection Bias)

- **Cell survival** after perturbation: you only observe cells that survived, creating selection bias
- **Quality control filters**: cells passing QC may have correlated features

### 1.12.3 Mediators

- **Pathway activation** mediates perturbation effects on phenotype
- **Transcription factor activity** mediates genetic variant effects

### 1.12.4 When Do-Calculus Helps

Do-calculus provides the formal framework to determine:

1. **Which covariates are safe to adjust for** (avoid colliders and their descendants)
  2. **When a mediator can rescue identification** (front-door criterion)
  3. **When observational data cannot identify the effect** (need experiments or additional measurements)
- 

## 1.13 10. Practical Checklist

When you encounter an interventional query  $p(\cdot | do(\cdot))$ :

1. **Draw the DAG** you believe represents the causal structure
2. **Check for back-door adjustment:**
  - Find a set  $Z$  that blocks all back-door paths from  $X$  to  $Y$
  - Ensure  $Z$  contains no descendants of  $X$

- If found:  $p(y | do(x)) = \sum_z p(y | x, z) \cdot p(z)$

**3. If back-door fails, check for front-door:**

- Find a mediator  $M$  such that:
  - $X \rightarrow M$  with no confounding
  - All effect of  $X$  on  $Y$  goes through  $M$
  - $X$  blocks confounding for  $M \rightarrow Y$

**4. If both fail:**

- The effect may be **non-identifiable** from observational data
- Consider: experiments, instrumental variables, proxy variables, or stronger assumptions

**5. Beware of colliders:**

- Never condition on a collider (or its descendants) unless you have a specific reason
  - Selection/survival variables are often colliders
- 

## 1.14 Summary

Do-calculus provides a complete, algorithmic approach to causal identification:

Concept	Key Idea
$do(x)$ operator	Graph surgery: cut incoming edges to $X$
D-separation	Determines conditional independence from graph structure
Rule 1	Insert/delete observations based on d-separation in $G_X$
Rule 2	Exchange actions and observations
Rule 3	Insert/delete actions
Back-door	Adjust for confounders that block back-door paths
Front-door	Use mediator to identify effect despite unmeasured confounding
Collider bias	Conditioning on colliders creates spurious associations

## 1.15 Further Reading

- Pearl, J. (2009). *Causality: Models, Reasoning, and Inference* (2nd ed.)
- Peters, J., Janzing, D., & Schölkopf, B. (2017). *Elements of Causal Inference*
- Hernán, M. A., & Robins, J. M. (2020). *Causal Inference: What If*

## 1.16 Next Steps

The natural continuation is to work through **identification by hand** for a realistic biology DAG—for example, with perturbation ( $X$ ), cell cycle ( $Z$ ), batch ( $B$ ), pathway activation ( $M$ ), and survival/selection ( $S$ )—showing exactly where each do-calculus rule applies and where identification fails.