

What are the sequence effects?

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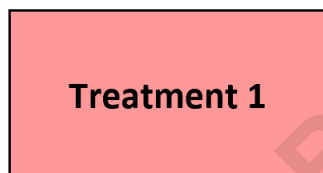
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In the realm of rigorous experimental design, particularly within repeated measures or within-subjects studies, researchers must meticulously account for how the administration of one treatment might influence the perception or performance of a subsequent treatment. A critical methodological pitfall in this context is the sequence effect. A **sequence effect** occurs when the specific order or arrangement of experimental treatments given to participants causes those treatments to interact with each other, fundamentally altering the measurement outcome of later conditions.

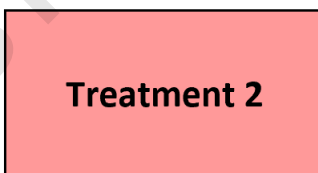
Unlike simple practice or fatigue (which constitute order effects), the sequence effect is characterized by a specific interaction: the nature of Treatment A changes the participant's response to Treatment B, often disproportionately or asymmetrically. Recognizing and mitigating these interactive influences is paramount to ensuring the internal validity and reliability of research findings. This comprehensive tutorial delves into the precise definition of sequence effects, provides detailed examples across different experimental domains, and outlines scientifically proven methods, such as counterbalancing and temporal separation, to minimize their impact on data integrity.

The Sequence Effect

Performance during this treatment affects the next treatment.



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The core challenge presented by the sequence effect lies in its potential to confound results. If researchers fail to control for these interactions, they risk attributing observed differences in participant behavior or measurement scores solely to the intended independent variable, when in reality, the results are artifacts of the procedural order. Understanding the subtle yet crucial difference between this phenomenon and generalized order effects is the first step toward achieving methodological sophistication in complex experimental designs, ensuring that the collected data accurately reflect the hypothesized causal relationships rather than unintended procedural side effects.

Defining the Sequence Effect in Experimental Design

A sequence effect, often described as a differential carryover effect, specifically concerns the interaction between adjacent or temporally close experimental conditions. This effect is distinct because the change is not caused by the simple accumulation of time or repeated exposure; rather, it is the **specific content, difficulty, or magnitude** of the preceding condition that biases the assessment of the current condition. For example, exposure to an extremely positive stimulus can set a high internal benchmark, making a subsequent neutral stimulus appear relatively negative, even if its objective neutrality remains constant. This bias is known as a contrast effect, a common manifestation of sequence problems.

In quantitative research, this effect poses a severe threat to the interpretability of data derived from repeated measures designs, where the same participants are exposed to all levels of the independent variable. The goal of such designs is efficiency and control over individual differences, but this benefit is compromised if the presentation order systematically changes the nature of the stimuli themselves. The existence of a strong sequence effect often implies that the treatments cannot be considered independent events; instead, they function as a chain reaction, where the state induced by one condition influences the perception or outcome of the next. Thus, researchers must adopt robust methodological safeguards to isolate the true treatment effect from these procedural interactions.

This phenomenon is widely studied in fields ranging from psychophysics and cognitive psychology to consumer research. The common thread among all sequence effects is the establishment of an internal reference point or anchor. When participants are asked to make judgments, perform tasks, or provide ratings sequentially, their assessment is frequently relative to the most recent condition they experienced. This psychological anchoring leads to systematic biases, where conditions are either over- or under-estimated based on the contrast, assimilation, or habituation induced by the immediately preceding condition. Understanding this mechanism is key to developing effective mitigation strategies, primarily focusing on breaking the cognitive or perceptual link between successive trials.

Distinguishing Sequence Effects from Order Effects

While related to the timing of treatment administration, the terms sequence effect and order effect describe distinct threats to internal validity. Understanding the precise difference is vital for applying the correct statistical or procedural controls, such as appropriate counterbalancing.

An **Order effect** refers to the generalized changes in performance that occur simply due to the position of a treatment within the sequence, irrespective of the specific content of the treatments involved. These are systemic, time-dependent changes that affect all participants similarly across different experimental tasks. The two most common forms are **practice effects**, where participants improve because they become more familiar with the task mechanics, and **fatigue effects**, where

performance declines due to mental or physical exhaustion incurred during earlier tasks. If participants systematically get better or worse at a task over time, regardless of whether they received Treatment A followed by B or Treatment C followed by D, this constitutes a general order effect.

Conversely, the **Sequence effect** (or differential carryover effect) occurs when the specific characteristics of one treatment condition interact with and alter the impact of the subsequent condition. For instance, if exposure to a high-dose stimulant (Treatment A) dramatically affects the participant's reaction time in a subsequent cognitive test (Treatment B), the influence is content-specific. The key distinction is that order effects are typically additive and symmetrical (affecting performance linearly over time), while sequence effects are interactive and often asymmetrical, meaning the specific content of A's influence on B is unique and not mirrored in a general time-based effect.

Contextualizing Sequence Effects: Carryover Effects

To fully appreciate the mechanism of the sequence effect, it is helpful to understand the broader category of carryover effects. A **carryover effect** describes any lasting influence that a particular experimental condition has on a subsequent condition. This encompasses all residual impacts, whether physiological, psychological, or perceptual.

The sequence effect is typically classified as a **differential carryover effect**. This term highlights that the magnitude or direction of the carryover is not uniform across all condition pairings. For instance, the sequence of Treatment A followed by Treatment B might produce a profound shift in participant response, whereas Treatment B followed by Treatment A might produce a negligible or entirely different shift. Because the carryover effect is contingent upon the specific preceding condition, it necessitates rigorous control methods that specifically address these asymmetric interactions, not just generalized time-based improvement or decline.

Therefore, when designing experiments, researchers must predict and differentiate between these threats. If the primary threat is generalized fatigue, simple balancing of time across conditions might suffice. If the primary threat is a strong, asymmetrical interaction between specific treatments--the sequence effect--then more sophisticated methodological solutions, such as the Latin Square or complete counterbalancing, are required to ensure that the effect of the sequence is isolated and minimized across the dataset. Failure to account for differential carryover effects can lead to invalid conclusions about the efficacy or nature of the experimental treatments.

Examples of Sequence Effects in Cognitive and Perceptual Tasks

The clearest domain where sequence effects manifest is in tasks involving subjective judgment, rating scales, or comparative assessment, where the participant establishes an internal anchor

based on immediate prior experience. The following examples illustrate scenarios where interactive carryover effects undermine the measurement accuracy.

1. Quiz Difficulty Assessment (Contrast Effect)

Suppose researchers ask participants to take five different math quizzes (Q1 to Q5) and assess their perceived difficulty after each completion. In this within-subjects experimental design, the difficulty level encountered in a previous quiz is highly likely to affect the subjective rating of the current quiz. For example, if a participant takes an **extremely difficult quiz** for Q1, and is immediately followed by a **moderately difficult quiz** for Q2, they may rate Q2 as "easy" or "manageable." They are using Q1 as their immediate anchor point, leading to a strong contrast effect that biases the Q2 rating downwards, despite Q2 being objectively challenging. The sequence (Difficult followed by Moderate) fundamentally altered the perception of the moderate condition.

2. Assessing Weight (Sensory Adaptation)

Consider a study where researchers ask participants to assess the weight of three different dumbbells, one immediately after the other. In this psychophysical task, the weight experienced in the previous lift establishes a momentary sensory threshold known as adaptation. If a participant first picks up a **20-pound dumbbell** and then a **10-pound dumbbell**, they might incorrectly think the 10-pound dumbbell is much lighter than its true weight. Their muscles and sensory receptors have adapted to the high exertion of the 20-pound weight, creating a powerful contrast effect. This is a classic example of a sequence effect stemming from immediate sensory carryover effect, where the objective measurement is skewed by the context of the preceding stimulus.

3. Assessing Speed (Perceptual Anchoring)

In a similar manner, perceptual biases manifest when assessing speed. Suppose researchers ask participants to assess the velocity of four different sprinters, sequentially viewing video clips. If the first sprinter (S1) is exceptionally fast, the participant establishes an extremely high reference point for speed. When the participant views S2, a moderately fast athlete, they are likely to judge S2 as slower than their true pace relative to the general population. The specific velocity of the previous sprinter directly affects the participant's internal rating scale, skewing their perception of the current sprinter and demonstrating how the sequence of treatments can corrupt subsequent measurements.

Strategy 1: Mitigating Sequence Effects through Temporal Separation

One primary methodological approach researchers use to minimize the interactive nature of sequence effects is to increase the temporal distance between the administration of experimental

treatments. This strategy aims to allow the participant's psychological or physiological state to return to baseline before they encounter the next condition.

Researchers can implement a **washout period**, also known as a resting interval or recovery phase, which is specifically designed to dissipate the residual effects of the preceding treatment. The goal is to break the sensory or cognitive linkage between trials. For example, instead of making participants assess the weight of dumbbells immediately, researchers could provide 10 minutes in between each assessment. This interval provides sufficient time for the muscle exertion and sensory adaptation induced by the heavy dumbbell to subside, allowing the participant's baseline perception of "heaviness" to reset, thereby minimizing the contrast or assimilation effect on the subsequent rating.

The effectiveness and necessary duration of the washout period are highly dependent on the nature of the experimental manipulation. In studies involving pharmacological agents, the washout period must be long enough for the drug to be fully metabolized and cleared from the participant's system--potentially days or even weeks. In cognitive studies, a few minutes of distraction, rest, or engagement in an unrelated filler task might suffice to break the cognitive linkage or anchor established by the previous trial. By carefully spacing out the time between experimental treatments, participants are more likely to provide responses that reflect the true effect of the current treatment, rather than responses that are contaminated by the specific context of the previous exposure.

Strategy 2: Employing Counterbalancing Techniques

While temporal separation is effective for physiological or immediate sensory effects, the most robust statistical defense against sequence effects (and order effects) involves strategic manipulation of the treatment order through counterbalancing. **Counterbalancing** is a fundamental methodological control technique in repeated measures experimental design, where researchers assign experimental treatments in different orders to different groups of participants.

The core principle of counterbalancing is to ensure that, across the entire cohort of participants, every condition appears equally often in every sequential position (first, second, third, etc.). More importantly, for controlling differential carryover effects, it ensures that every condition is preceded and followed by every other condition an equal number of times. This systematic variation allows researchers to statistically isolate the true treatment effect from the systematic influence of the sequence. For example, if researchers have three conditions (1, 2, 3), they would strive to use all six possible permutations (123, 132, 213, 231, 312, 321) an equal number of times across the study sample.

By ensuring that each possible order is used the same number of times across the sample, researchers effectively distribute the impact of the sequence effect--whether it is contrast,

assimilation, or differential carryover--evenly across all treatment conditions. The influence of the order then becomes part of the error variance rather than a systematic bias, allowing the researcher to average the measured results across all sequences and obtain a cleaner, more accurate estimate of the true mean difference attributable solely to the treatments themselves.

Advanced Counterbalancing Designs for Complex Studies

While complete counterbalancing (using all $n!$ sequences) is the gold standard for controlling for all sequence interactions, it quickly becomes impractical as the number of experimental conditions (N) increases. For instance, five conditions require 120 unique sequences, demanding a prohibitively large sample size. Consequently, researchers frequently rely on partial counterbalancing methods that balance only the most critical sequence properties.

The most common and effective technique for managing complex designs while controlling for first-order sequence effects is the **Latin Square Design**. In a Latin Square, the number of required sequences is drastically reduced to N (the number of conditions), where each condition appears exactly once in each sequential position. Crucially, a well-constructed Latin Square ensures that every condition precedes every other condition exactly once. This partial balancing efficiently controls for the primary threat: first-order differential sequence effects, which represent the influence of the immediate preceding trial.

However, it is vital to note that partial counterbalancing, such as the Latin Square, only controls for the interaction between adjacent treatments. It does not account for second-order sequence effects (where the sequence A-B affects the perception of C differently than B-A affects C) or complex interactions spanning multiple trials. Therefore, the choice of counterbalancing strategy must be guided by a thorough theoretical understanding of the experimental variables and the potential magnitude of the anticipated sequence effect.