The effect of acute itch on the motor evoked potential: An investigation using transcranial magnetic brain stimulation

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Abstract

Past research into the functioning of the brain during itch has revealed significant activity in the motor cortex, however, the role of the motor cortex during itch is not completely known. It is theorised to be involved in the planning of scratching movement. Two studies compared the effect of histamine induced itch and placebo on motor evoked potentials, elicited from navigated transcranial magnetic stimulation from the first dorsal interossei muscle. Study 1 investigated cortical changes in the contralateral motor cortex. Study 2 investigated the ipsilateral motor cortex. For both studies, neurotypical participants had to do a simple visual attention task whilst receiving pulses. Motor evoked potentials were recorded from 16 neurotypical participants in study 1, then baseline corrected peak-to-peak amplitudes were analysed across conditions. There was a significant increase in baseline corrected amplitudes for the histamine condition compared to the placebo within the contralateral motor cortex. Furthermore, analysis showed significant amplitude increases 5 to 8 minutes post prick, compared to the first 1 to 4 minutes across conditions. For Study 2, 14 participants' data was analysed. There was only a significant difference between halves, with the last 5-to-8-minute half showing significantly higher amplitudes than the first. It was concluded histamine itch sensations tend to elicit higher levels of excitability, especially in the contralateral motor cortex. However, the arousal from the attention task may have also facilitated this increase in excitability.

Introduction

Pruritus or itch is an unpleasant, localised, or generalised, skin sensation that triggers the urge to scratch (Taylor et al., 2010). The urge to scratch in evolutionary terms serves the purpose to remove an irritant, such as a mosquito or poison ivy from the skin, therefore the nervous system has evolved to encourage such an urge (Sanders et al, 2019). Regarding actual scratching, its function is to provide a pleasurable mild pain to relieve itchiness (Paus et al., 2006) because the pain inhibits itch sensations (Ikoma et al., 2006). From this, scratching creates a two-fold reward: relief from itch sensations and a hedonic experience (Sanders et al., 2019). Therefore, scratching provides a functionally antagonistic role to reduce itch and shows the compulsive-desire dimensions of itch. However, in terms of dermatology, this relationship between itch and scratch can be disturbed. Patients diagnosed with atopic dermatitis are aware they should resist the temptations to scratch, unfortunately scratching bouts reinforce the urges induced by pruritus (Sanders et al, 2019). This creates a vicious cycle of itch addiction, rather than provide relief and inhibit itch sensations, scratching exacerbates it (Ishiuji, 2019). Additionally, this leads to sensitisation in itch neural pathways causing hyperknesis (perceived itch stimuli is amplified) and alloknesis (gentle stimuli being perceived as itchy) (Ishiuji, 2019). Ishiuji (2019) highlighted how the urge to scratch and the salience of receiving hedonic pain are akin to drug cravings. Considering this, if people affected by this cycle refrain from scratching, it may lead to inhibition of such an urge, eventually leading to a break in the cycle.

Past research has often used histamine to induce itch experimentally (Schut et al., 2015). Usually, a 1% histamine solution droplet is applied to the skin, which is then pricked with a sterile lancet to allow a miniscule amount to enter the upper epidermal layer. Such a procedure leads to an itch sensation, which peaks approximately around 120 seconds (Darsow et al., 2000). Usually a flare (superficial skin reddening) and wheal (small oedema at the skin prick site) skin response are seen (Darsow et al., 2000). Another way is via cowhage spicules applied to the skin, however, cowhage does

not cause flare, but instead induces a burning and pricking pain sensation (Papoiu et al., 2011; Papoiu, et al., 2012).

The corpus of neuroscientific research shows histamine induced itch sensations stem from specialised and unmyelinated C-fibres from cutaneous nerve endings which send action potentials to the dorsal root ganglia and spinal cord. From there, the impulse is projected by the ventrocaudal nucleus medialis to the anterior cingulate cortex (ACC) and dorsal insular cortex (DIC) (Paus et al., 2006). Functional magnetic resonance imaging (fMRI) and functional positron emission tomography (fPET) imaging correlated activations in the "Itch matrix" consisting of the anterior insula, cingulate cortex, as well as the primary somatosensory cortex (S1), premotor cortex, prefrontal cortex, thalamus, and cerebellum. (Drzezga et al., 2001; Holle et al., 2012; Hsieh et al., 1994; Mochizuki et al., 2003). It is theorised that each functionally specialised member of the network signifies polymodal (sensory, motor, and emotional) processes involved in itch, interdependent with each other (Holle et al., 2012). The ACC and anterior insular cortex (AIC) may play an important role in the emotions of pruritus and form an urge to scratch. This theory stems from research that shows these areas are pivotal in the processing of affective components of pain (Ikoma et al., 2006), the genesis of affective physiological and behavioural reactions, and the influence of motivational salience in response to external stimuli (Medford & Critchley, 2010). Somatosensory cortical activity represents the sensory processing of itch, furthermore, coactivations in the motor areas, it is argued, may reflect the preparation and planning to scratch (Holle et al, 2012), such a response is a spinal reflex modulated by these top-down motor areas (Sanders et al., 2019).

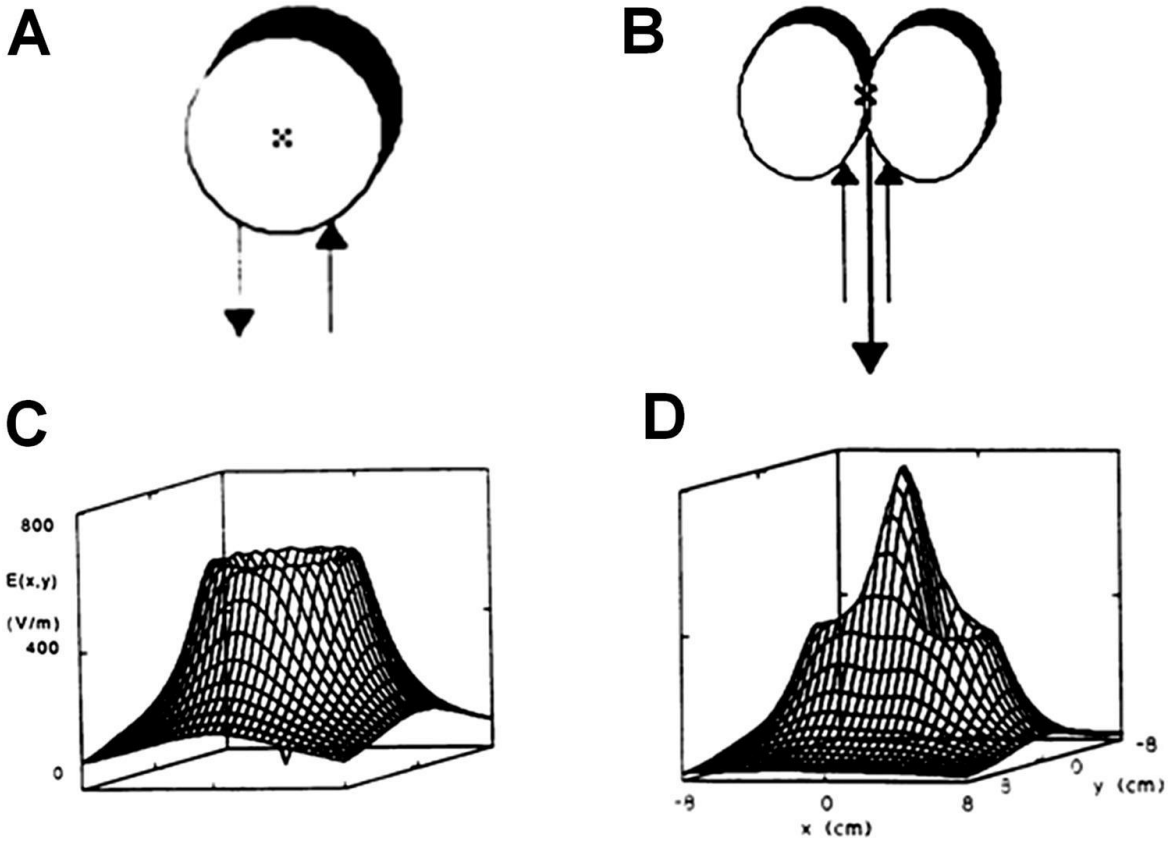
However, it must be considered there are significant commonalities and differences between the neuroscience and psychology of itch and pain. Pain and itch are undoubtedly different subjective experiences that induce different reflexes, pain evokes the withdrawal reflex and itch the scratch reflex (Paus et al., 2006). Research using neuroimaging comparing the activations between pain and itch showed similar activations in the areas mentioned before. However, these data also revealed no

activations in the secondary somatosensory cortex (S2) during itch, but a higher activity in ipsilateral motor areas during itch, comparatively to pain (pain showed higher activity in the contralateral areas) (Ikoma et al., 2006). Therefore, it can be theorised that increased activity in the motor areas ipsilateral to the side of itch play a pivotal role in the genesis of scratching planning in response to itch, thus the limb moving to the affected site (whilst pain triggers withdrawal from the affected limb, thus showing a decrease in activity) (Ikoma et al., 2006). Furthermore, research highlights stronger activations in the prefrontal and orbitofrontal cortices which are involved in decision making, reward and hedonic systems (Paus et al., 2006), as well as inhibiting negative emotion processes in the amygdala (Sanders et al., 2019). However, the functional roles of the itch matrix are not completely understood, as most of these functions have been based on their processes during pain, their specific purpose during pruritis is comparatively less known (Jones et al., 2018) Firstly, most research on the functional specialisations involved in itch utilise neuroimaging, whilst such methods excel in temporal and spatial data, it is only correlational. Therefore, the causal role of a region in processing the polymodal dimensions of itch cannot be concluded (Jones et al., 2018). Some studies have used transcranial magnetic stimulation (TMS) as an independent variable to investigate the causal roles of regions in the itch matrix.

Transcranial Magnetic Stimulation

TMS is a type of non-invasive brain stimulation, which can inhibit or excite a localised part of the brain by producing a short (100μs) magnetic field to that area. This magnetic field comes from a magnetic coil, which is also applied to the scalp. When the coil is activated, it creates a magnetic flux running through the coil, which leads to an electrical field perpendicular to the magnetic field. Usually, the electricity will cause a looping flow of current inside the brain which is parallel to the position of the coil. Coils have a crosshair at the centre of its plane. Therefore, any neurons parallel to the looping current are then depolarised (Halett, 2007). The shape of a coil is normally a circle, a figure-of-eight, or a cone (see Figure 1), however different shapes have different stimulation characteristics. Figure-

of-eight coils emit the highest currents at the middle intersection, so they provide a focused stimulation to a target area (Halett, 2007). Increasing the intensity of the stimulation increases the current which deepens the effect of stimulation (Rossi et al., 2021). However, the characteristics of these coils means they lack the capability to induce depolarisations at greater cortical depths (Zangen et al., 2005).



Note. Referenced from Hallet, M. (2007).

Figure 1.

Diagram Showing a Circular and Figure-Of-Eight Coil and their electrical fields.

There are various methods of TMS stimulation. Single-pulse TMS is often used to explore functional roles of specific brain regions, for example applying a single-pulse over the motor cortex. Whereas paired pulse stimulation (2 pulses with an interval of 2 to 100s of milliseconds between) can be utilised to assess cortical inhibition of target areas, or across hemispheres. Repetitive TMS on the other hand can induce measurable neural changes to a target area after stimulation. (Klomjai et al., 2015).

From an ethical viewpoint, application of TMS on neurotypical participants is very safe. However, it is possible to unintentionally induce a seizure, which is considered the most severe effect from TMS. Genesis of seizure occurs when many neurons are triggered en masse at once. For example, as of 2020, 8 seizures were induced from single pulse TMS, however current data does not quantify out of how many. There are many important factors that can increase the risk of seizure; hence it is vital potential participants are screened for these, by doing so, the probability of seizure significantly lowers. History and/or diagnosis of epilepsy must be screened for, but also other neurological and psychiatric illnesses can increase risk. For example, neurological damage from stroke to Alzheimer's, as well, evidence has shown populations with depression, bipolar and schizophrenia have an elevated risk of seizure. Additional factors include sleep deprivation, significant levels of stress and anxiety, and alcohol consumption. Interestingly, it used to be believed single pulse TMS had a lower risk of seizure induction compared to rTMS paradigms, however, data shows there is no significant difference between these types of stimulation (Rossi et al, 2021).

It is also important researchers are correctly trained in administering TMS safely and effectively. Fried et al (2021) recommended training should contain teaching the fundamentals of TMS, such as the key theory on how it works, how to conduct TMS ethically and safely in tandem with international to institutional regulations. Then, training specifically of TMS protocol, such as screening participants to reduce the risk of seizure mentioned prior

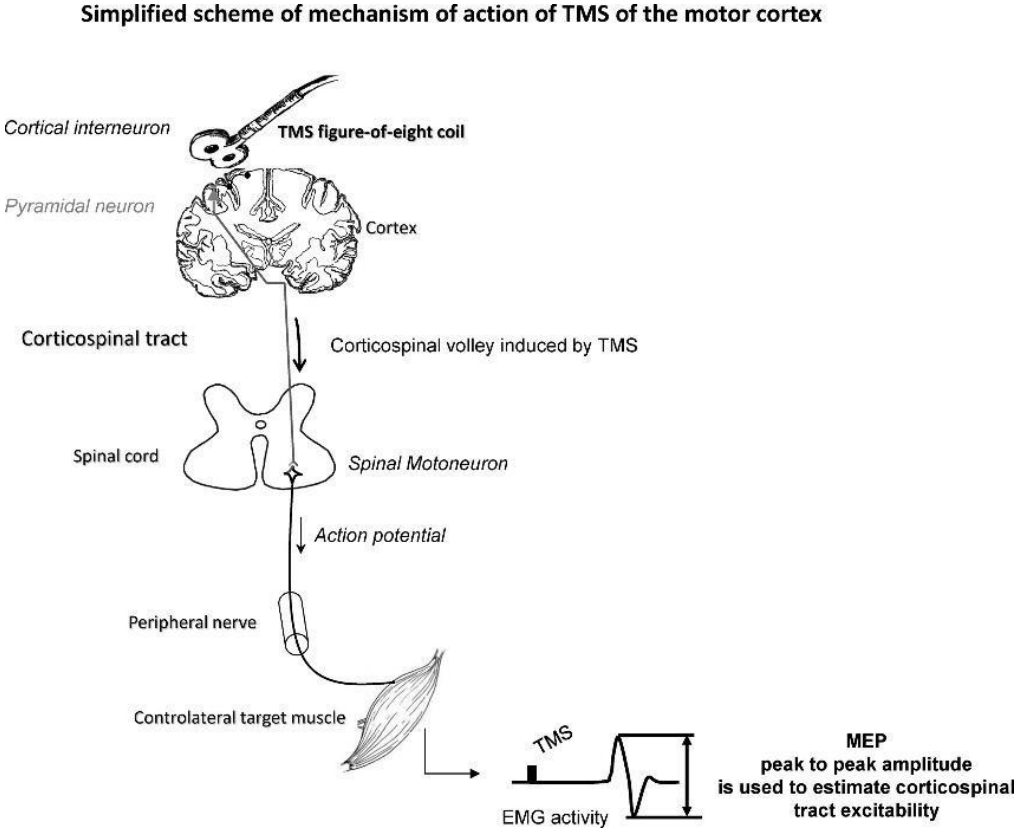
Regarding related research, Jones et al (2018) used repetitive magnetic pulses to the S1, secondary somatosensory cortex and inferior frontal gyrus to investigate their causal role in itch by inhibiting cortical excitation, leading to a reduction in itch intensity. Stimulation to the S1 led to a significant decrease in itch ratings although, S2 and IFG stimulation did not yield significant reductions on ratings. Jones et al (2018) proposed the S1's causal role was in processing the sensory discriminative dimensions of itch. Interestingly, the researchers put forth explanations for the results: TMS reduces cortical input from the afferent c-fibres responsible in histamine induced itch, thus leading to reduced pruritic intensity. On the other hand, TMS leads to a disruption of top-down processes involved in pruritus.

Motor Evoked Potentials

There have been many studies which used TMS to measure and assess the motor cortical excitability of the motor cortex. By stimulating the motor cortex with a single pulse, motor evoked potentials are elicited as a result, which are recorded via an EMG, from a skin electrode attached to a muscle belly (Hallet, 2007; Klomjai et al., 2015). A motor-evoked potential (MEP) is a succinct muscle response elicited by stimulation over the primary motor cortex (see Figure 2) (Hallet, 2007). In general, increased TMS stimulation leads to an increase in MEP amplitude (until a plateau (Wehahn et al., 2007)) and reflects the strength of cortico-spinal projections and the facilitation of motor systems in the brain (Dai et al., 2016; Wehahn et al, 2007). A single focused pulse activates pyramidal tract neurons (PTN) within the primary motor cortex; therefore, excitability of the PMC will determine the amplitude of the MEPs (Wehahn et al., 2007). This is because these neurons, which connect to premotor areas in the brainstem and spinal cord, are heavily involved in motor planning and execution (Economo et al., 2018). Different intensities will induce different cortico-spinal neural waves. For example, low intensity stimulation with a figure-of-eight coil in a posterior anterior direction induces an I1 wave, which is a neural wave stemming from indirect activations of PTNs. Whereas higher

intensity will eventually lead to a direct stimulation of PTNs, termed D-waves (Di Lazzaro & Ziemann, 2013).

Resting motor threshold (RMT) is often a vital factor for motor cortical assessment, RMT is defined as the minimum intensity to elicit MEPs greater than 50uV five out of ten trails (Klomjai et al., 2015). MEP peak-to-peak amplitudes are then analysed to assess excitation and inhibition and represents how many pyramidal tract neurons were triggered from TMS pulses (Pellegrini et al., 2018). Another, more detailed way, is to assess the direct relationship between MEP amplitude and TMS intensity through stimulus-response MEP curves. These represent input and output of the motor cortex, or on the graph, to show the relationship between MEP amplitude and TMS intensity (Wehahn et al, 2007).



Note. Taken from Klomjai et al (2015).

Figure 2

Diagram showing the pathway of a TMS elicited MEP.

Previous research on measuring TMS elicited MEPs has shown evidence for motor cortical, and spinal inhibition, contralateral to the side of muscle pain. These studies recorded muscle activity of the right hand via EMG, then induced muscle pain to the same hand, which led to a reduction in average MEP amplitude. (Le-Pera et al, 2001;Burns et al, 2016). Svensson et al (2003) found cortical inhibition lasted for more than ten minutes after pain. Furthermore, suprathreshold stimulation 125% of a participant's resting motor threshold elicited the most significant reduction in MEP amplitudes, compared to other TMS intensities (Svensson et al, 2003).

There has been no current research on the effect of the urge to scratch on the amplitudes of MEPs. As stated before, pain and itch have a psychological and neuromechanical relationship, pain leads to the withdrawal reflex, and data shows motor cortical inhibition contralateral to the side of pain. It is currently unknown whether induction of acute itch in a muscle will lead to contralateral motor cortical inhibition, similarly to what is observed in pain. Blood Oxygenation Level Dependent (BOLD) imaging data did find that refraining from scratching led to increased activity in the contralateral inferior central gyrus, which is responsible for motor movement inhibition (Kleyn et al., 2012). Likewise, it is unknown if itch applied opposite to the EMG recorded hand may result in ipsilateral motor cortical excitation to the side of itch. On the other hand, if it will lead to inhibition reflecting participants' refrain from scratching, and so the inhibition of the urge to scratch, as refraining led to significant decreased activity in the ipsilateral motor cortex (Kleyn et al., 2012).

The aim of the two studies were to investigate the causal role of the motor cortex which theoretically processes the urge to scratch and measure possible motor inhibition and excitation. To answer the gaps of knowledge in how motor cortical inhibition or excitation is affected by the urge to scratch, two studies were conducted. Study 1 investigated how the contralateral motor cortex changed in excitability during itch sensations. Whilst Study 2 investigated how the ipsilateral motor cortex changed in excitability during itch. The independent variable was the skin prick test, where a

histamine solution (to induce itch) or a placebo solution (as a control) was applied to compare differences in peak-to-peak MEP amplitudes. The dependent variable was motor evoked potentials elicited by TMS, which are recorded by EMG. For the first study, where EMGs were recorded from the right first dorsal interossei (FDI) muscle, and itch was induced on the same muscle, it was hypothesised that TMS-evoked MEPs should show significant inhibition in amplitudes, based on findings from pain research. For the second study, where EMGs were recorded from the right FDI muscle, and itch was induced on the opposite FDI muscle, it was hypothesised TMS-evoked MEPs should show significant change in amplitudes (either increases, reflecting planning of the urge to scratch, or decreases, reflecting inhibition of the urge to scratch).

General Method

Participants

An a-priori power analysis indicated that to detect a significant effect of Prick type with an effect size of Cohen's d= 0.8 and an 80% probability in a within-subject design, a sample size of 15 participants was required for each study. In total, across both studies, 46 participants were contacted and took part in at least one session.

For Study 1, 16 right-handed, normal, or corrected-to-normal participants (5 male and 11 female); aged 18-49 (M = 25.5) took part in this study. 29 were contacted, however, 6 did not complete both TMS sessions. Additionally, 7 were included in MEP analysis, however, analysis revealed 4 had significant EMG noise so their data was unusable. Also, 3 were excluded because of error in counterbalancing conditions (i.e., received two placebo skin pricks).

For Study 2, 14 right-handed, normal, or corrected-to-normal participants (7 male and 7 female); aged 20-36 (M = 27) took part in this study. 20 were contacted and participated in both sessions, however, 6 were excluded because of EMG noise, therefore their data was unusable.

Participants either received course credit or were paid at a rate of 8 pounds per hour. All gave full written informed consent and were fully debriefed at the end of their participation. The study was approved by the University of Hull's School of life sciences Ethics Committee and conducted in accordance with the ethical guidelines of the ethical declaration of Helsinki 2.

Apparatus and Stimuli

Transcranial Magnetic Stimulation

An appropriately sized 10 20 EEG cap was worn by participants. For TMS, a Duostim 70mm figure-of-8 coil was used to administer single pulse TMS. The coil was placed firmly on the scalp, above the motor cortex contralateral to the hand from which EMG was recorded, to elicit MEPs in the FDI. The coil was angled 45 degrees to the sagittal plane, so that the pulse would travel in a posterior-anterior direction/cortico-motorneurone direction. EMG data were recorded from two disposable electrodes, in a belly-tendon montage. Adhesive tape was also applied to the sides of the electrodes to ensure they remained secure and to reduce EMG noise. Brainsight neuronavigation software was used throughout to track the coil's orientation to measure degrees of error and ensure consistency.

Attention Task

A simple attention task was presented to participants from a computer monitor, participants had to silently count how many red squares flashed on the centre of the screen. They were positioned approximately 70cm away from the monitor, the monitor had a 24inch display. They were required to sustain their attention on the task for the entirety of stimulation. Blue or red squares would flash for 0.5 seconds, the order of square stimuli was randomized (see figures 4, 5 and 6). The computer was connected to the Duostim TMS machine and the Brainsight computer, ensuring stimulation, coil tracking and the task were synchronized. Psychopy was used to create the task and script the stimulation participants received during it. The task was comprised of two blocks, the baseline (3 minutes) and post-prick block (8 minutes). The task began with a screen requiring the input of the necessary information, such as participant number. The next screen informed the participant of the task (see figure 3). All trials began with a white central fixation cross against a gray background, TMS pulses were externally triggered by the script, every 7. 5 seconds, whilst Brainsight recorded the orientation of each pulse.

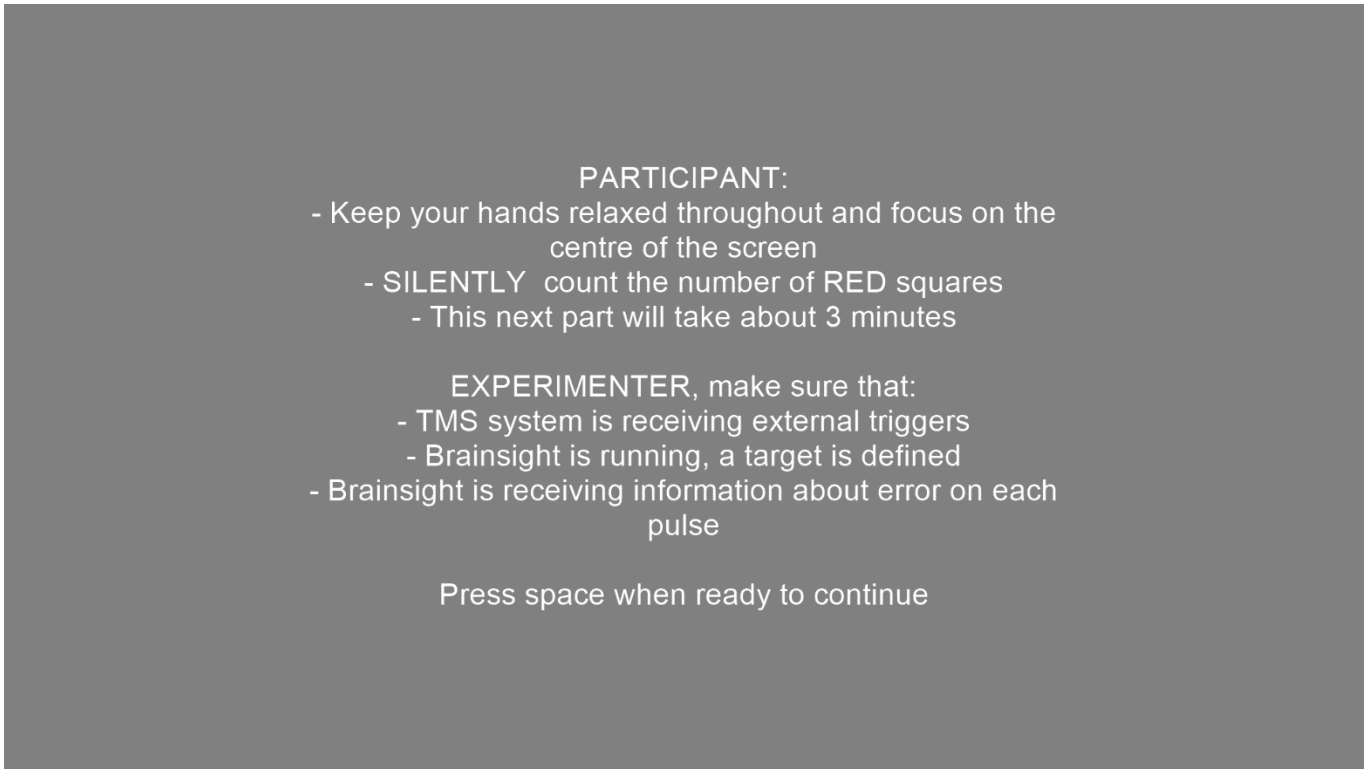


Figure 3.

A figure of the instructions participants and the experimenter had to follow before the task begin.

Figure 4.

The fixation cross participants had to focus on whilst stimuli were presented to them.

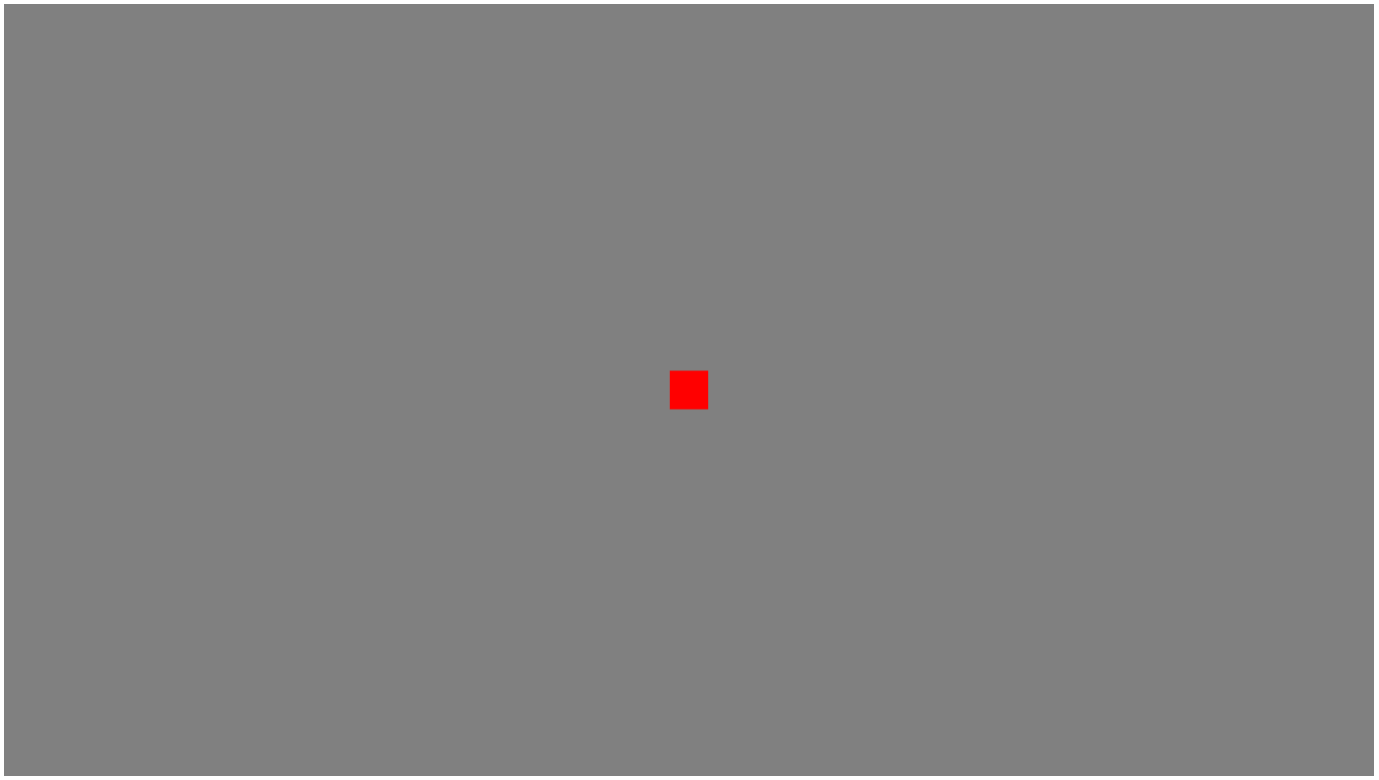


Figure 5.

A frame showing the red square stimuli.

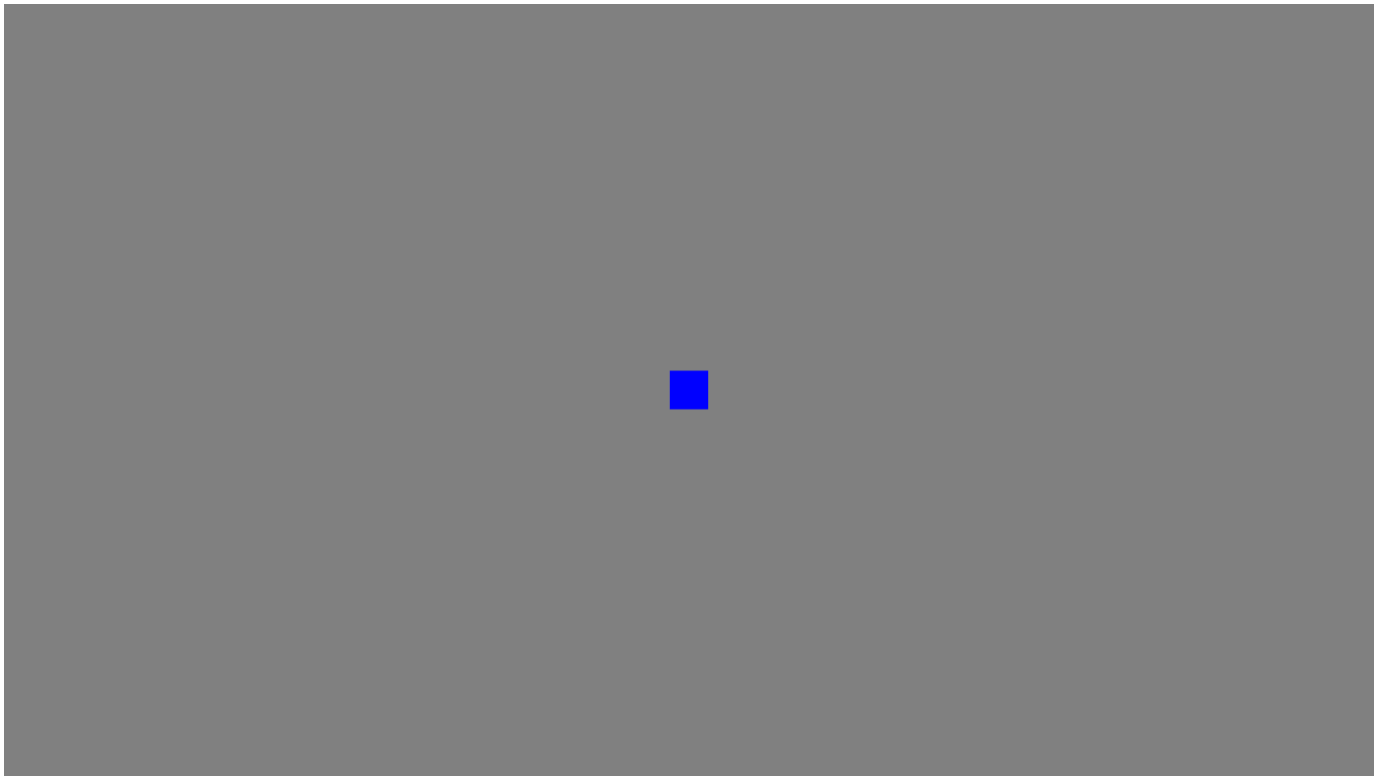


Figure 6

A frame showing the blue square stimuli.

Itch Induction

Acute pruritis was induced using a histamine skin prick procedure. A 1% histamine dihydrochloride aqueous solution droplet (~50 µl) was given near the right or left FDI andsubsequently, the skin was superficially pricked by a 1mm tip of a lancet (see appendix). The prick was to ensure a minimal amount of histamine solution entered the upper epidermal layers of the skin. An itch sensation usually began approximately 35 seconds after entering the skin, which peaks in intensity around 120 seconds post prick. Diminishing of the sensation typically takes 30 to 60 minutes. Regarding the placebo control condition, a pure aqueous solution was used.

Ethical Considerations

Although past literature has emphasised the safety of TMS, there still was a small chance stimulation could induce a seizure in neurotypical participants. To address this, all participants had to complete and sign a TMS screening questionnaire and a Histamine prick screening questionnaire (see appendix). The exclusion criteria included a history of skin conditions, histamine intolerance, and any history of seizures/epilepsy, neurological and psychiatric disorders. Participants were additionally required to abstain from consuming alcohol above three units on the preceding day to the study, as well as foregoing it completely on the day in question. Participants were also requested to avoid consumption of any recreational drugs, as well as refraining from drinking coffee in the hour leading up to the study. All researchers received the appropriate training concerning how to follow TMS safety protocols. Furthermore, in case of an emergency, all researchers received first-aid training. In addition to the Histamine Prick Test Questionnaire screening, all researchers were trained on how to safely administer the itch stimulus. Informed consent was provided by all participants and their right to withdraw was emphasised at the beginning of the study. Participants' data was anonymised, and participants were debriefed on the purpose of the study, the types of stimulation they would receive, and informed of both the anonymisation of their data as well as their right to withdraw.

Design/MEP Analysis

Participants had to partake in both conditions for a full set of data. Each study required two sessions. In one session, participants received a histamine skin prick to their hand after a baseline of cortical excitation was recorded. In the other session a placebo skin prick was given after the baseline. The hand used for skin prick was counterbalanced across participants, but within participants, the same hand was pricked in both sessions. For the first study, the prick was applied to the same hand where EMG was recorded, for the second, the skin prick was applied to the contralateral hand relative to EMG.

Statistical Methods

The experimental design was a 2 x 2 mixed factorial, where Prick Type (histamine vs placebo) was manipulated as a within-subject variable, whereas the hand to which the prick test is applied to (either left or right) was realized as a between subject manipulation. To assess changes in cortical excitability during conditions, baseline corrected peak to peak amplitudes were calculated by taking the mean amplitude of the pre-prick stage for that session, then calculating the percentage difference between that and the mean amplitudes from the post-prick stage. Baseline-corrected peak-to-peak amplitudes of the MEPs were binned into two four minutes half bins. These data were subjected to a 2 x 2 repeated measures ANOVA, with the within subject variables Prick Type (histamine vs placebo) and Time (first half (1-4 minutes) vs second half (5-8 minutes) post skin prick). If the data violated the assumption of normality, then a series of Wilcoxon signed rank tests was used for analysis. MATLAB scripts converted MEP data into millivolts, which was then put through a 30-2000hz filter. Another script plotted each MEP trial to assess evidence of pre-pulse muscle contraction and the amplitude of the MEP. Additionally, all trials were organised into their respective sessions where mean and standard deviations of the pre- pulse and MEP were calculated. Evidence of muscular contraction was defined as all trials where the pre-pulse peak-to-peak amplitude was more than 2 standard deviations above that subject's mean pre-pulse peak-to-peak amplitude. Any participants with fewer than 50% of trials in any half bin after applying this outlier rejection were excluded from the analysis.

Procedure

Localisation of the M1

Once the participants provided informant consent and passed the TMS and Histamine screening, they were seated in front of a desk, aligned to the centre view of the monitor. A file for each participant was created which saved MEP data. Participants were given the appropriately sized EEG cap; the CZ electrode position was aligned to the vertex. Such a position was calculated by half the

distance between the inion and nasion. The electrodes to the target hand were applied, the participants hand remained relaxed to ensure a clear electromyogram (EMG) signal. EMG were recorded from the first dorsal interosseus (FDI) in a belly-tendon montage contralaterally to the side of TMS. Once ready, a combination of methods was used to localise the M1. The starting location to find the M1 from the CZ position was 4cm laterally, 1cm anterior (FC3). To further localise the participant's primary motor cortex, MEPs were tested 1cm anterior to the generic target, 1cm laterally, 1cm posterior, and 1cm medially, in a grid-like pattern. If a certain spot elicited stronger MEPs from stimulation, this became the centre region, and the grid pattern was repeated. The specified area to elicit MEPs in the target muscle the most was marked with a small sticker on the EEG cap. Furthermore, the location was recorded using Brainsight neuronavigation software. A tracking headstrap was fastened around the EEG cap to track the position of the participant. The participant's seating position was adjusted so they were in centre of the tracking camera's field of view, to avoid any desynchronization issues.

Resting Motor Threshold

The resting motor threshold (RMT) was then determined, RMT was classified as the minimum intensity to elicit 5 out of 10 MEPs in the FDI muscle (an MEP was defined as a peak-to-peak amplitude difference of 0.05mV). The participant's RMT was recorded in the Duostim software.

Recording of MEPs and Attention Task

125% of the participant's resting motor threshold was calculated and set on the TMS machine, then the attention task was run. The experimenter typed in the necessary information such as bottle number. Once Brainsight ensured the coil was aligned correctly over M1, the attention task script was executed, which externally triggered TMS pulses. They received single supra-threshold TMS pulses for 3 minutes, with 7.5 second intervals on average between pulses, to record a baseline whilst following the attention task. In-between the baseline and post-prick blocks, participants were asked how many red squares they counted, then received a skin prick (either histamine or placebo) near the muscle belly of the hand. The task post-prick block was executed, where participants

received single TMS pulses for another 8 minutes, also with an average time of 7.5 seconds between pulses. Participants were asked to refrain from scratching. Throughout data collection, the EMG was monitored for evidence of muscle contraction and/or interference.

Itch Response

Both skin prick type conditions were administered in a double-blind manner. At the end of TMS stimulation, participants were asked if they experienced itch or not, indicated by a question on the monitor (the experimenter explained to the participant how to respond using the keyboard). The next question asked participants to rate the intensity of itch at the prick site, on a scale of 0 to 4, with 0 indicating “no itch” and 4 indicating “intense itch”. Lastly, the skin was inspected by a research assistant or the participant for the presence of skin reactions (wheal and/or flare). Wheal is defined as a small dermal oedema at the site of the skin prick, and flare is the superficial skin reddening around the site of the prick. These measurements were used to assess the subjective and physiological factors of pruritis, and to help determine if the itch stimuli were administered correctly.

Results

Study 1

A Kolmogorov-Smirnov test was used to assess normality of baseline corrected peak to peak MEP amplitudes across conditions. There was a significant value for histamine second half $D(16) = .23$, $p=.03$, indicating that the data significantly deviated from a normal distribution. Therefore, non-parametric tests for all subsequent analysis were used.

On average, baseline correct MEP amplitudes were higher in the second half across conditions compared to the first half. Specifically, the baseline corrected MEP amplitude was higher for the placebo second half (M=122.95, Mdn=114.09, SD=38.64) compared to the placebo condition first half (M=100.99, Mdn=100.1, SD=33.88). The average corrected amplitude was higher for the histamine second half (M=135.42, Mdn=118.34, SD=37.74) compared to the histamine first half (M=116.23, Mdn=103.70, SD=34.91) (See Figure 7 and 8).

It was first tested whether MEP amplitudes significantly changed relative to the baseline period through a series of Wilcoxon signed rank tests. These tests indicated that MEP amplitude was significantly increased during the second half of the post-prick phase, both for the histamine condition (Z =3.41, p =.001) as well as the placebo condition (Z =2.12, p =.034). During the first half of the post-prick phase, MEP amplitudes for did not differ significantly from baseline, for both placebo condition (Z =-.26, p =.80) and histamine condition (Z =1.35, p = .18).

Next, the conditions were directly compared against each other through a series of related samples Wilcoxon Signed Rank tests. When collapsing over time, there was a significant positive difference between the placebo and histamine condition, z=-1.97, p=.049, indicating greater MEP amplitudes for the histamine condition (*Mdn* = 106.75) than for the placebo condition (*Mdn* = 104.32). When collapsing over prick type, there was a significant difference between halves, z=-3.10, p<.05, indicating greater MEP amplitudes during the second half (Mdn = 118.34) than during the first halve (Mdn = 100.68) of the post-prick phase. To test for possible interaction between prick type and time, a new variable was created that captured the difference between halves across conditions

[(histamine_1st_half – placebo_1st_half) - (histamine_2nd_half – placebo_2nd_half)]. To assess for a potential interaction, a one sample Wilcoxon Signed Rank test was then applied on this difference variable with a test value of 0. Analysis showed there was no significant interaction between prick type and time Z=.05, p=.96.

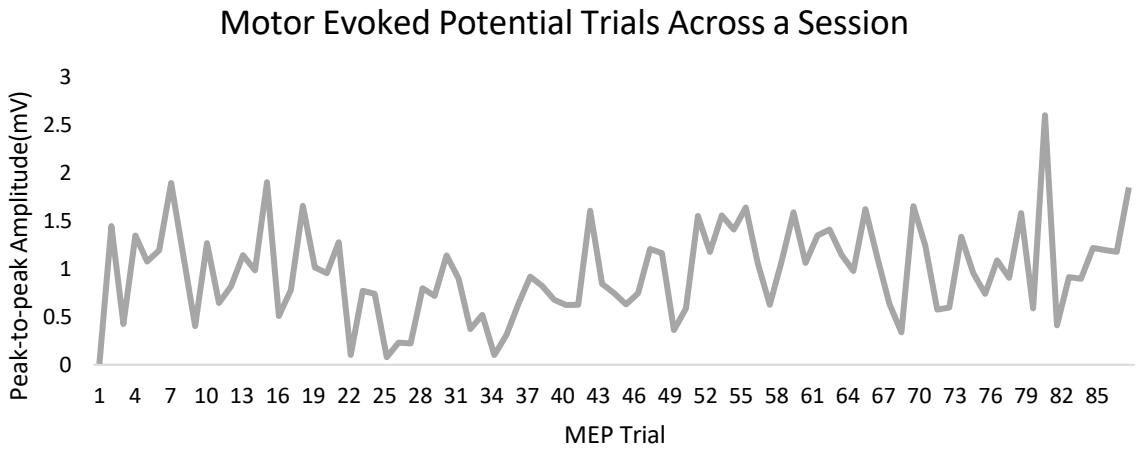


Figure 7.

Example of MEP amplitude variability across trials for one session of a single participant

Study 2

To assess cortical excitability the same data analysis method was conducted for the second study. Baseline corrected peak to peak amplitudes were calculated by taking the mean amplitude of the pre-prick stage for that session, then calculating the percentage difference between that and the mean amplitudes from the post-prick stage. To compare baseline-corrected MEP amplitudes between studies see figure 8.

A Kolmogorov-Smirnov test was used to assess normality of baseline corrected peak to peak MEP amplitudes across conditions. There was a significant value for placebo second half $D(14) = .25$, $p=.02$, indicating that the data significantly deviated from a normal distribution. Therefore, non-parametric tests for all subsequent analysis were used.

On average, baseline correct MEP amplitudes were higher in the second half across conditions compared to the first half. The baseline corrected MEP amplitudes were higher for the placebo second half (*M*=108.403, *Mdn*=100.90, *SD*=37.47) compared to the placebo condition first half (*M*=93.47, *Mdn*=90.14, *SD*=26.82). The average amplitude was higher for the histamine second half (M=114.46, *Mdn*=109.422, *SD*=22.19) compared to the histamine first half (*M*=102.94, *Mdn*=104.19, *SD*=17.674).

A Wilcoxon Signed Rank Test indicated that MEP amplitude was significantly increased during the second half of the post-prick phase for the histamine condition (Z =2.04, p =.04), but not for placebo condition second half (Z =.28, p =.78). During the first half of the post-prick phase, MEP amplitudes did not differ significantly from baseline, for both placebo condition (Z =-1.48, p =.14) and histamine condition (Z =.53, p = .59).

Next, the conditions were directly compared against each other through a series of related samples Wilcoxon Signed Rank tests. When collapsing over time, there was no significant difference between the placebo and histamine condition, z=-1.16, p=.25, indicating no MEP amplitude differences for the histamine condition (*Mdn* = 108.70) and the placebo condition (*Mdn* = 100.75). When collapsing over prick type, there was a significant difference between halves, z=-2.54, p=.01, indicating greater MEP amplitudes during the second half (*Mdn* = 111.24) than during the first halve (Mdn = 98.20) of the post-prick phase. To test for possible interaction between prick type and time, a new variable was created that captured the difference between halves across conditions

[(histamine_1st_half – placebo_1st_half) - (histamine_2nd_half – placebo_2nd_half)]. To assess for a potential interaction, a one sample Wilcoxon Signed Rank test was then applied on this difference variable with a test value of 0. Analysis showed there was no significant interaction between prick type and time Z=-.66, p=.51.

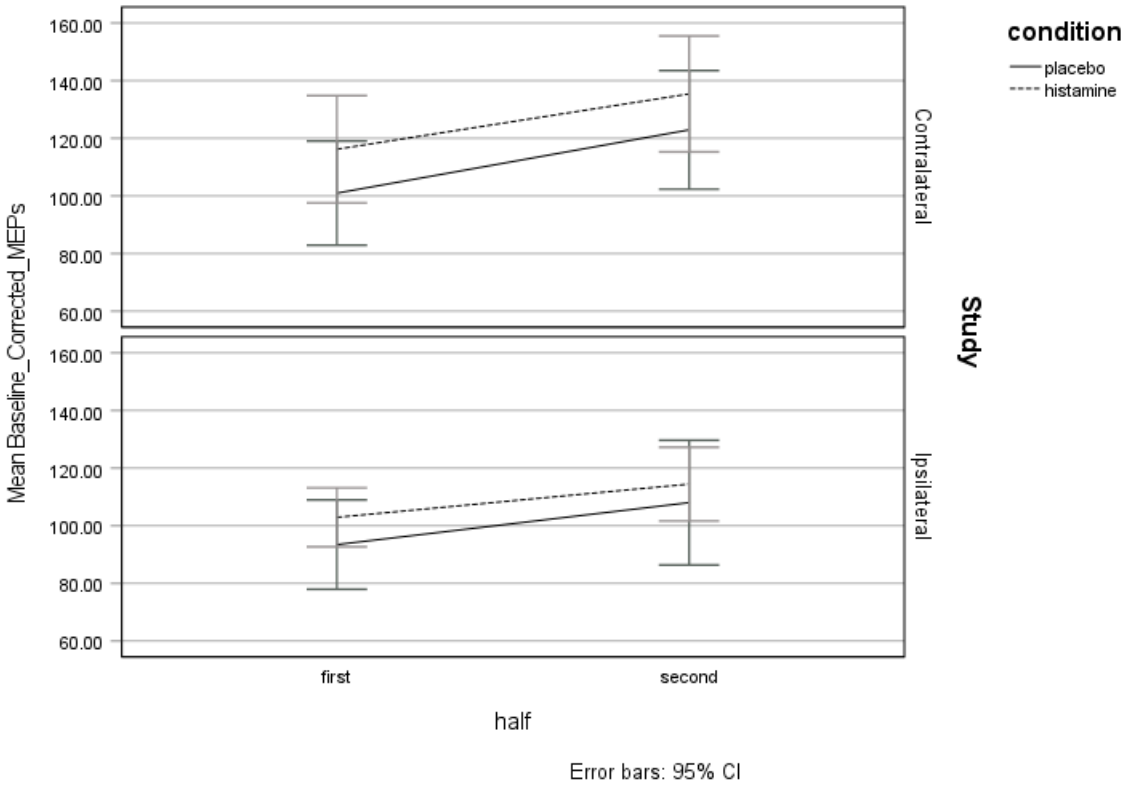


Figure 8.

Multiline graph to compare baseline corrected amplitudes between the two studies across halves.

Discussion

For both studies, participants received navigated single pulse TMS over the motor cortex to elicit MEPs during a pre-skin-prick and a post-skin-prick phase, to assess the differences in cortical excitation. Itch was induced through a histamine solution, or they received a placebo. For the first study, analysis revealed a significant difference in baseline correct amplitudes between the placebo and histamine condition, specifically on average TMS elicited higher amplitudes across histamine conditions compared to placebo conditions. As well, there was a significant difference between halves for both conditions, where the latter half elicited higher amplitudes on average comparatively to the former half. For study 2, no significant difference was found between histamine and placebo conditions, however there was a significant difference between histamine halves, where the latter half elicited higher amplitudes compared to the former.

Study 1 – The Effect of Itch Contralaterally to the Motor Cortex

The aim of Study 1 was to investigate changes in cortical excitability in the contralateral motor cortex to the side of itch, participants received a skin prick on the same hand as the EMG. It was hypothesised that for the ipsilateral condition, MEP amplitudes may have decreased during the histamine condition compared to the placebo, thus indicating motor cortical inhibition. Such a hypothesis was based on evidence that because itch and pain share a significant neurophysiological relationship, the same pattern would occur like it has been shown during pain. Albeit the results indicated motor cortical excitation occurred, especially during the latter 4-8 minutes half after the skin prick.

There is ample evidence to show motor area activity during itch, however the functional role is not known. Brain imagining data shows motor cortical activations contralateral to the side of itch (Ishiuji et al., 2009). One theory is that these increases in MEP amplitudes signify the motor cortex becoming excited to plan scratching movement. The motor cortex is extremely adept at planning movements in response to sensory information. Movement is often fast, therefore planning (if

participants are given sufficient time to do so) is vital for improving accuracy and speed (Svoboda & Li, 2018). It was hypothesised by the researchers that the affected limb will not want to move, based on the pain withdrawal reflex, and how evidence shows contralateral motor cortex inhibition to the side of pain. The data here shows inhibition certainly does not occur. This contrasts somewhat with BOLD evidence showing refraining from scratch led to significant increased activity in the contralateral inferior central gyrus (which is involved in movement inhibition) (Kleyn et al., 2012). However, increases in this region may not necessarily mean/lead to motor cortical inhibition in the contralateral motor cortex.

It is clear itch evokes the scratch reflex (Ikoma et al., 2006), typically the opposite limb moving to the affected site on the opposite side. The evidence of excitation here may show that the reflex occurs contralaterally to the side of itch. In behavioural terms, this may mean that the affected limb is poised to move (to get rid of itch sensations) possibly to assist the other arm to scratch or the affected arm wants to move regardless of what the other limb is meant to do. Arguably, this is not surprising, Sanders et al (2019) summarises well how the nervous system is highly evolved to scratch. The salience of an irritating itch sensation, and how neuroimaging data shows striatum and limbic system activations, which are responsible for reward and motivation increase in activity (Rinaldi, 2019), clearly means humans are extremely sensitive from a cortical to behavioural level in wanting to get rid of the sensation regardless of where itch comes from on the skin. Rinaldi (2019) summarises humans much prefer to experience mild pain over itch.

However, it is important to consider the neuro-cognitive impact from the contralateral somatosensory cortex during planning scratch behaviours. Said previously, the S1 and S2 are theorised to process the temporal spatial sensory factors of itch (Jones et al., 2018). Sensory perception is vital for conscious behaviour and functioning, so the brain needs to process where the itch sensation stems from. An in-vivo study on itch and scratch in mice found S1 neurons encoded motor planning information before the onset of scratching behaviour (Chen et al., 2022). As well, the precuneus within

the somatosensory cortex (which is theorised to process subjective itch and pain sensations) may facilitate changing one's attention to the itchy sensation and induce imagining moving their hand to scratch (Mochizuki & Kakigi, 2015). Evidence shows strong contralateral M1 and S1 activations in planning finger movements, specifically, S1 coritcomotorcortical neurons connect to motor neurons that control muscle receptors, thus the S1 plays a significant role in movement planning (Arian et al., 2022). From this, processes from the somatosensory cortex may help in motor cortical excitation, and the planning of the urge to scratch, considering it plays a pivotal role in processing where the itch is, draw attention to it, and thus aid the motor cortex in scratch planning.

Alternatively, the recorded excitability can be explained by the effects of attention and arousal during experimentation, rather than effects from itch. For example, studies found significant increases in cortical excitability before the stimuli were presented (for example, just observing a screen's background and remaining still) (Hannah et al., 2018). As well, attentive focus on hand muscles can induce excitability, which can occur at a purely motor cortical level (no such effect was found on a spinal level from h-reflex data) (Ruge et al., 2014). This is important considering the experimenter encouraged attention on the hand by reminding participants to keep their hands relaxed throughout, that their hand will twitch from pulses, and will receive a skin prick on the hand. Therefore, motor cortical excitation may have already occurred before the skin prick began. Furthermore, evidence shows attention to a visual task will also increase MEP amplitudes (Ruge et al., 2014). The attention task for this experiment was to avoid boredom and to ensure participants remained focused and relaxed throughout stimulation. So not only may there be a possible effect from focus on the hand, but arousal from the task may have also increased excitability, which can explain the increase during placebo conditions as well. Albeit the data still showed a significant difference between placebo and histamine. Possibly the arousal from the experiment and the urge to scratch induced from itch led to an extremely excited state within the motor cortex. The implications of this means the motor cortex, contralateral to the site of itch, plays some role. However, it is interesting there was no evidence for inhibition whatsoever. If it is planning scratching, arousal or a combination of both, the evidence

shows there may not be a withdrawal reflex present. Meaning the hand does not withdraw and remain still as a protective mechanism from further pain. However, it cannot be concluded with complete confidence it is significantly responsible for the planning of scratch. If the increased amplitudes indicated planning to scratch then the hand, in this study became poised to move to get rid of this sensation.

Study 2 – The Effect of Itch Ipsilaterally to the Motor Cortex

The aim of Study 2 was to investigate changes in cortical excitability in the motor cortex ipsilaterally to the side of itch, participants received a skin prick to the opposite hand of the EMG recorded hand. It was hypothesised that either MEP amplitudes during itch compared to placebo would lead to a significant increase (indicating motor cortical excitation and the urge to scratch), or a significant decrease (indicating inhibition, from refraining to scratch). This hypothesis came from research that showed activity in the motor cortex during itch, however the causal role of this activity is not known. The results indicated that there was a general increase in MEP amplitudes in the histamine condition compared to the placebo however this was an insignificant difference. Albeit there was a significant difference between the second and first half within the histamine condition, specifically the latter half evoking higher MEP amplitudes than the former.

Although there was no significant excitation or inhibition between histamine and placebo, it can be theorised that the data supports the theory that planning to scratch is processed in the ipsilateral motor cortex. Essentially, the opposite hand to the side of itch is poised to move to scratch the affected site. Neuroimaging data does show premotor area activations during itch ipsilaterally to the side of itch and as mentioned previously, comparing data between pain and itch showed analogous activity, however motor cortical activity appeared during itch, which was absent during pain (Ikoma et al., 2006). The present study's data somewhat contrasts with BOLD evidence that found refraining from scratch led to decreased activity within the ipsilateral precentral gyrus (the location of

the motor cortex) (Kleyn et al., 2012). The lack of effect between conditions therefore might be because the ipsilateral motor cortex is extremely sensitive to planning to scratch regardless of itch sensation. However, histamine conditions across halves showed higher MEP amplitudes compared to placebo. Perhaps this is due to the motivational salience of potentially removing the itch sensation. Mochizuki et al (2014) found that actual scratching deactivated the primary motor cortex and anterior cingulate cortex (the region involved in emotional and motivational aspects of itch). So, during histamine conditions, and if participants experienced a placebo effect, the motor cortex was still motivated to remove itch sensations.

An alternative, although not exclusive explanation, as discussed in Study 1, is that participants knew the study focused on itch, attention was drawn towards the hand and/or arousal, moderated by the experimental task, might have been the significant facilitator of motor cortical excitability. Hence there still were amplitude increases during placebo conditions. This effect was even more pronounced during histamine conditions; the average amplitudes were even higher. Still, apart from the placebo first half condition, there was no evidence of inhibition. The lack of significant effect on itch though means it is hard to discern exactly what the ipsilateral motor cortex's role is during itch.

The lack of effect from itch might be due to MEP variability. As illustrated in Figure 8, there is considerable variability from one trial to the next in participants' MEP amplitudes. This trial-to-trial variability of MEP amplitudes and its test-retest reliability have also been discussed in the literature. Even when coil orientation and intensity are controlled for, a pulse can elicit noticeably different amplitudes, which is caused by factors such as amplification noise to changes in excitability in the underlying neurons (Goetz et al., 2019). Additionally, there is evidence of increasing age of participants, especially female, usually leads to higher MEP variation comparatively to males (Pitcher et al., 2003). This may have had some effect for both studies, considering recruitment did not obtain a gender balance and there was a notable range of ages across the sample. A way to assess/compare this study and future studies' variability can be through statistical models. Goetz et al (2019) has

produced a MEP amplitude model which generates virtual participants and their MEPs based on what TMS intensity they received, and outputs the expected variability.

Moreover, the study's interpulse interval (IPI) for pre and post prick was on average 7.5 seconds, range 5 to 10 seconds. Literature shows an IPI within this range elicits good reliability between trials and sessions, however, pulses at 10 seconds tended to elicit higher amplitudes compared to 4 seconds (Pellegrini et al., 2018). The explanation being cerebral blood perfusion (rate at which blood from the arteries is delivered to a region's tissue) normalises 10 seconds post pulse (Pellegrini et al., 2018). The IPI range used for this study might have unintentionally encouraged variability in amplitudes, as longer or shorter IPIs would have then elicited different amplitudes. Variability and factors mentioned before, means it can be difficult to assess if changes in MEP amplitudes are a valid reflection of cortical excitability (Pellegrini et al., 2018). Although histamine conditions in general lead to increased amplitudes, there still was considerable variation across conditions which make it difficult to interpret how much of an effect the itch conditions had.

General Discussion

Study 1 aimed to investigate contralateral motor cortical inhibition during itch sensations. Study 2 aimed to investigate if itch would lead to excitation or inhibition within the ipsilateral motor cortex. Both studies found motor cortical excitability, especially during the histamine condition and second half. It was previously unknown what the causal role of the motor cortex was during itch, and it was theorised the ipsilateral motor cortex was responsible for planning the urge to scratch. Overall, inhibition was not found for both the contralateral and ipsilateral motor cortex. Interestingly, Study 1 found higher amplitudes of excitation compared to study 2. On the one hand, it can be theorised the main process of planning to scratch comes from the contralateral motor cortex. Whilst the ipsilateral motor cortex might be comparatively less excitable, and plans to move the hand to scratch, regardless of itch or placebo conditions.

Regarding cognition, many studies, like this one, required participants to not move, however imagining movement before it happens can activate the motor cortex (Najafi et al., 2020). Study 1 and 2 found a significant increase in MEP amplitudes for the last half of the placebo condition. This can be explained by a cognitive element. Participants were aware they took part in an itch-scratch study; however, they may have imagined scratching and/or became more aware of itch sensations on other parts of the body, leading up to excitation across the last 4 minutes. This can only be theorised; however, Humans are extremely sensitive to the suggestions of itch. Past research has shown that viewing itch-based stimuli or listening to a lecture on itch led to increased scratching behaviours compared to controls (Lloyd et al., 2013; Niemeier & Gieler, 2000). Viewing scratching activates itch-matrix regions such as the the S1 and PMC (Holle et al., 2012). Furthermore, a study found verbally labelling the itch stimuli participants received as high intensity led to significantly higher levels of reported itch (van Laarhoven et al., 2011). The present study only asked participants to measure when they felt the site of the prick was at its most intense, so it is unknown if they became more aware of itchy sensations, pre and post skin prick. Regardless, there are other possible theories to explain excitability during itch sensations that may not be purely the motor cortex planning to scratch.

It was hypothesised that refraining from scratching, especially for Study 1 would lead to motor cortical inhibition, the implication being it can help understand the itch-scratch cycle and to aid future research investigate how to break it. Populations who cannot escape the cycle experience a significant effect on motor control, they cannot resist moving to scratch (Ishiuji, 2019). Based on this, if one refrains from scratching, inhibition may not occur, instead motor cortical planning to scratch may only end when the sensation is gone, or attention is drawn away. Meta-analysis from Najafi et al (2020) showed that decreasing itch sensations led to changes in activity such as the thalamus and the bilateral ACC, however no changes were found in the supplementary motor area (SMA). Whilst the SMA is a different cortical region to the M1, meta-analysis was unable to specify cortical changes in the M1 because of inconsistencies in methods across studies. Moreover, the data supports evidence that activations of the reward system encourage activity in the SMA and PM, which comes from

motivations to move cause by reward expectancy (Mochizuki & Kakigi, 2015). Then it is no wonder people affected by atopic dermatitis succumb to the itch-scratch cycle. They would have to tolerate an annoying itch sensation and resist the urge to scratch that will not be inhibited by refraining alone. Perhaps then, treatment that focuses on reducing itch sensations might be impactful in breaking the cycle. For example, naltrexone cream (an opioid receptor antagonist) can be applied to the affected and skin helps reduce itch sensations after 15-30 minutes, which can last up to 6 hours (Bigliardi et al., 2007). Therefore, by alleviating the sensation, this will reduce the urge to scratch.

Limitations

Both studies used neuronavigation software to track the coil's degrees of freedom during experimentation. The purpose, mentioned before, was to ensure consistent coil orientation over the motor cortex in a posterior-anterior direction. The orientation will affect which underlying circuits are depolarised from a pulse, posterior-anterior recruit early I-waves, whilst lateral-medial recruit d-waves (Pellegrini et al., 2018). Therefore, consistent orientation will hopefully reduce amplitude variability. Some literature has shown that navigated TMS can elicit higher MEP amplitudes and improve replicability of participants' RMT by reducing RMT variation (Jung et al., 2010). However, one study argued using navigation methods do not help circumvent the issue of amplitude variability. Statistical analysis of MEP amplitude differences between non-navigated and navigated conditions found no significant difference. Interestingly, standard deviations and coefficient of variances (to measure variability) increased as stimulation intensity increased and these measures also showed no reduction in navigated conditions (Jung et al., 2010). Coil orientation was controlled for, so the underlying neuromechanisms involved in an MEP must be the significant factor in variability. Jung et al (2010) discusses how there is variability in how many alpha motorneurons (spinal neurons that connect to peripheral nerves that lead to muscle contraction (Squire et al., 2013)) are recruited during a pulse, and fluctuations in these neurons' excitability. There seems to be no possible way to

circumvent this, regardless, navigation during this experiment proved to be extremely useful, coordinates of the hotspot were saved and then used to find the participants RMT, ensuring reliability and accuracy in trying to determine their true resting motor threshold.

Supra-threshold stimulation was 125% for all participants, to replicate Svensson et al's (2003) study which elicited the highest level of inhibition during pain compared to other intensities. A benefit of using this intensity is that data shows supra-threshold stimulation 120%-135% significantly decreases amplitude variability, thus improving test reliability (Pellegrini et al., 2018). But a notable limitation of this study was it did not assess MEP amplitudes at different supra-threshold intensities due to resource restraints, unlike Svensson et al's (2003) study. Mentioned in the introduction, different intensities depolarise different pathways within the motor cortex, such as only higher intensities directly activating cortical pathways, leading to higher elicited amplitudes. So, participants who received higher suprathreshold stimulation in general elicited higher amplitudes compared to lower suprathreshold participants. For example, lower threshold participants' stimulation only indirectly depolarised motor cortical pathways (Pellegrini et al., 2018). Pellegrini et al (2018) states how many studies rely on 120% suprathreshold data to assess cortical excitation, whereas utilising a range of intensities will better reflect the effect of TMS on the brain region. Therefore, it might be fruitful to investigate how different suprathreshold intensities might affect amplitudes during itch in future research, which would also help determine the intensity that elicits the optimal level of excitation during the experiment.

Lastly, it may be reductive to conclude these studies show a purely cortical level of excitability. MEPs record a top-down summation of cortical to spinal to neuronal excitability, thus excitation may occur at a spinal-peripheral level. For example, the scratch reflex does not occur purely at a cortical level, it is a spinal reflex modulated by top-down motor processes (Sanders et al., 2019). Likewise, Ruge et al (2014) demonstrated motor cortical excitation at a cortical level, but also used h-reflex data to assess it at a spinal level. These studies did not use this; therefore, these studies were unable to

determine if this is the case. The implication is that determining the causality of itch and what excitation occurs in this experiment is limited to a motor cortical level, thus was unable to ensure it is solely a top-down pathway of excitability. The studies were unable to demonstrate if cortical changes at a peripheral and/or spinal level contributes to motor cortical outputs. It might be fruitful for future studies to control for the effects of spinal and peripheral inhibition to explicitly determine the motor processes during the urge to scratch.

Suggestions For Further Research

A salient factor in assessing cortical excitability is the effect of attention and arousal during tasks. It seems this is an inevitability (Ruge et al., 2014). Therefore, it might be useful to replicate the experiment without an attention task. Although this might make it monotonous for participants, perhaps TMS might elicit lower MEP amplitudes, from a possibly less excited motor cortex. Then if the factors of attention and arousal are reduced, it might make it clearer what the role of the motor cortex is during itch. Furthermore, Svensson et al (2003) used a range of suprathreshold intensities to elicit MEPs and compare levels of inhibition. It might be prudent for future studies to also do the same to compare how different intensities might evoke higher or lower levels of excitability.

Both studies' data in general found noticeable excitation at a cortical level, but it will be helpful for future studies to also use h-reflex data to assess how itch sensations affect excitability at spinal to peripheral levels. Recent findings highlight how the Periaqeuductal Gray plays a significant role in processing top-down pathways, and imaging found significant activity during itch sensations (deactivations also occurred during scratching) (Mu & Sun, 2022). Therefore, it might be possible for future TMS, and h-reflex studies to investigate specifically what role the spinal level plays during TMS pruritis research. Based on evidence discussed before, the spinal level might also show significant excitation.

Navigated TMS for both studies proved to be extremely useful. It would be recommended for future studies to utilise navigation software, as it is particularly helpful in localising the motor cortex and determining participants' resting motor thresholds. A meta-analysis will be extremely useful to assess the efficacy navigated TMS has in reducing MEP variability between trials and participants.

Lastly, a way to assess/compare this study and future studies' variability can be through statistical models. Goetz et al (2019) provides an extremely useful statistical model to simulate MEP variability. This model generates virtual participants and their MEPs based on what TMS intensity they received, and outputs the expected variability. MEP variability is unavoidable, therefore researchers can compare the variation of amplitudes from their data with this model, to analyse if there are any significant abnormalities.

Previous imaging research showed bilateral activations in the motor cortex during itch. Which theorised its role was in planning to scratch. Whether the causality is itch sensations leading to motor planning scratching and/or the effects of attention and arousal, both studies found ipsilateral and contralateral motor cortical excitation, especially during histamine conditions. Interestingly, Study 1 found higher MEP amplitudes in the contralateral motor cortex compared to Study 2, it can be theorised this region might play a more substantial role than the ipsilateral equivalent. Additionally, a future study utilising h-reflex data and a range of suprathreshold intensities might help gain a better understanding of what the motor cortex's role is during itch.

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Appendix A

INFORMATION SHEET FOR PARTICIPANTS

YOU WILL BE GIVEN A COPY OF THIS INFORMATION SHEET

Title of study: The role of inhibitory and excitatory motor processes in planned scratch responses

I would like to invite you to participate in a research project which forms part of my ongoing research on the role of the motor system in itch. Before you decide whether you want to take part, it is important for you to understand why the research is being done and what your participation will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask me if there is anything that is not clear or if you would like more information.

What is the purpose of the study?

The purpose of the study is to study the role of the motor system when experiencing the urge to scratch. We hope to gain a better understanding of what gives rise to the intention to scratch an itch as the inhibitory processes that counteract acting out an urge to scratch.

Why have I been invited to take part?

You have been invited to participate in this study because you have indicated your interest on the SONA study information site, are healthy, and over 18 years of age.

What will happen if I take part?

A researcher will meet you to go over the information sheet and explain the procedures. The researcher will go through a Screening Form with you to make sure that it is safe for you to participate in the study. If you are happy to continue they will then ask you to sign a consent form. This study includes two visits to the TMS lab at the University of Hull. Each visit takes no more than 60 minutes, so the overall time commitment for this study is about 2 hours.

What is TMS?

Taking part in this study involves Transcranial Magnetic Stimulation (TMS). TMS is generally very safe, provided you meet the criteria of the medical screening. TMS is a technique that allows us to stimulate the brain by rapid switching of a magnetic field in a coil placed over the head. We can measure the effects of this stimulation by recording the activity of muscles (electromyography; EMG). EMG activity of the muscle is measured at the surface of the skin by attaching an electrode (small silver disc). Several electrodes will be taped on the skin over muscles on your hands for this purpose.

In the present study, we will use TMS to stimulate your brain using single pulse stimulation (known as single pulse TMS). Single pulses (separated by a few seconds will be applied over the scalp. At the same time, the activity in your muscles will be measuring using EMG or you may be asked to complete a task on the computer. You will be told to either contract or relax your muscles.

What do I have to do?

Before you take part in our study, we ask that you get a good night's sleep the night before, so that you are alert. Also, we ask you to refrain from excessive alcohol consumption (more than 3 units) the day before the study and any alcohol consumption on the day. We also ask that you refrain from use of recreational drugs before the study. You may drink coffee or tea as normal but we ask that you do not have a coffee for one hour before the study. If you are unsure about any of the above, please discuss these with the researcher before taking part.

Study schedule

Taking part in this study will involve an initial TMS session, to give you an idea what this form of stimulation feels like. You will receive detailed information about what taken part in the study involves, both in written and verbal form, and will be able to ask any questions you might have. We will then apply a skin prick test followed by single pulse TMS for a period of up to 10 minutes. A week later, you will be asked to come back for a second experimental session, where we will apply another skin prick test, followed by single pulse TMS for a period of up to 10 minutes.

If you choose to take part in the study, you will be asked to complete the medical screening questionnaires (one for TMS and one for the histamine prick test) and the consent form. The experimenter will be happy to answer any questions if you are not sure about any of the questions on any of these forms.

Database of regular TMS participants

If you decide to take part in this this study, we would like to invite you to become part of our pool of regular TMS participants. For this purpose, we ask on the consent form for your permission to store your contact details, so that we can contact you to let you know about upcoming TMS experiments in the future. Becoming a member of the database does not mean that you automatically agree to

take part in future studies; we will always send you all study information first so that you can make an informed choice.

If agree to become part of pool of regular participants, we will store your contact details in a safe place, for as long as you are a student at the University of Hull. After you have left the University, we will delete your contact details from the database. If you change your mind about the participant [then please let us know via email. We will then immediately delete all your contact details from the database. The following three people are conducting TMS experiments in the department and will have access to this database:

- Dr Henning Holle
- Dr Emmanuele Tidoni
- Dr Igor Schindler

Do I have to take part?

Participation is completely voluntary. You should only take part if you want to and choosing not to take part will not disadvantage you in any way. Once you have read the information sheet, please contact us if you have any questions that will help you make a decision about taking part. If you decide to take part, we will ask you to sign a consent form and you will be given a copy of this consent form to keep.

Payment/Incentives & benefit of taking part

If you need research participation credit for your Research Skill modules, you can receive 2 hours of course credit for taking part in this study. Should participation take longer than the planned two hours across the two sessions, you will receive additional course credit according to how much time you spent.

There is no other direct benefit for you in taking part in this study. Your results will however help to gain scientific knowledge on the neural processes underpinning acute itch.

What are the possible risks of taking part?

TMS-specific risks

TMS can sometimes cause a mild headache or muscle spasms, which invariably settle with time or with simple analgesics (common pain medication, such as paracetamol). Experimenters will observe you and question you to check that you are comfortable during the experiment. Prolonged, high intensity, high frequency TMS has very rarely been reported to induce brief seizures (less than 1 in 1000 studies). In most cases, the seizure was associated with a family history of epilepsy, existing neurological disease (e.g. multiple sclerosis) or medication (anti-depressant or dopamine

medication). The risk of a provoked seizure occurring in healthy individuals due to TMS is extremely small. As a precaution, you cannot take part in TMS if you have a personal or close family history of epilepsy. If you are taking any medication, you should discuss this with the researcher beforehand. If you suffer with migraine headaches, you should not take part in this study.

Risks associated with the histamine prick test

Taking part in the study will involve the experience of itch. The itch experienced after a histamine prick is similar to mosquito bite, although less intense. Itch and associated reddening of the skin have usually completely subsided 30 to 60 minutes after the application. The histamine prick test usually does not lead to bleeding. At most, there may be a droplet of blood. The experimenter has been trained in the correct application of the prick test.

When larger doses of histamine are infused directly into the blood stream, a number of unwanted side effects can occur. This side effects include headache, developing an itchy rash (urticaria), drop in blood pressure, constriction of the airways (bronchospasm) and cramp-like abdominal pains. In the present study, only a very small amount of histamine solution will be deposited in the upper skin layers of the test site (up to 2 µl, which is equivalent to 0.002 ml). Most of this small amount of histamine solution will remain at the site of skin prick, and very little (if any) will enter your blood stream. It is therefore extremely unlikely that you will experience any of the above-mentioned side effects. Nonetheless, we have to make you aware that these side effects exist.

In order to make sure that it is safe for you to take part, you have to answer a number of questions about your medical history and any medication you are currently receiving (please see the histamine prick test screening form for details).

In short, the test area, which is located in the middle of your forearm (see Figure on consent form), should be free from

- skin infections

- acute or chronic eczema

- signs of increased skin reactivity. Examples of very sensitive skin include skin that allows 'skin writing', or abnormally thick, dry or scaly skin

You also shouldn't be hypersensitive to any of the ingredients of the histamine solution, which are (apart from water and salt)

- Histamine
- Phenol
- Glycerol

- Sodium Hydroxide

You also shouldn't

- currently suffer from acute allergic symptoms
- suffer from a serious general disorder
- currently have a fever
- receive treatment with β-Blockers
- suffer from any disease of the heart or blood vessels (cardiovascular disease)
- have a history of low blood pressure
- have a history of fainting during medical procedures (e.g., during a flu shot, or immunization shot)
- suffer from asthma
- be pregnant or breastfeeding
- have taken antihistamines in the last 48 hours

Some people suffer from a condition called histamine intolerance. When eating histamine-rich foods (e.g., spinach, sauerkraut, certain types of sausage and fish), histamine-intolerant people tend to develop 'allergy-like' symptoms such as headaches, rashes, itching, diarrhoea, and vomiting or abdominal pain. If you suffer from histamine intolerance, you should not take part in this experiment (as a precautionary measure). If a histamine-intolerant person undergoes a histamine prick test, it may take longer than 30 – 60 minutes until the reddening of the skin has completely subsided.

No other risks of the histamine prick test are known to the investigator at this time.

Data handling and confidentiality

In this research study we will use information from you. In particular, this will be your personal data (name and contact details, and your individual brain scan) and the research data (the reaction time data). Your data will be processed in accordance with the General Data Protection Regulation 2016 (GDPR). We will only use information that we need for the research study. We will let very few people know your name or contact details, and only if they really need it for this study.

Everyone involved in this study will keep your data safe and secure. We will also follow all privacy rules. We will make sure noone can work out who you are from the reports we write.

With your agreement, your personal data (name and email address) will be stored in a secure database. In this database, we will also add a random identification code next to your name. All your other data (the recorded EMGs) will only contain that random identification code, but not your real name or other information that could identify you. Thus, in the unlikely event that an external person should get access to the research data, they will not be able to identify you, since these data have been anonymized.

The only people who will have access to the database with your contact details will be the Primary Investigator of this study, Dr Henning Holle, as well as Dr Igor Schindler and Dr Emmanuele Tidoni from the Department of Psychology. Your personal data will not be shared with any third parties. Your research data will be used to support current and future research and may be shared anonymously with other researchers.

We will store the anonymized research data for a period of 10 years. We will store the written consent forms for a period of 6 months. We will store your contact details for as long as you are a student of the University of Hull.

Data Protection Statement

The data controller for this project will be the University of Hull. The University will process your personal data for the purpose of the research outlined above. The legal basis for processing your personal data for research purposes under GDPR is a 'task in the public interest' You can provide your consent for the use of your personal data in this study by completing the consent form that has been provided to you. Information about how the University of Hull processes your data can be found at https://www.hull.ac.uk/choose-hull/university-and-region/key-documents/data-protection.aspx

You have the right to access information held about you. Your right of access can be exercised in accordance with the General Data Protection Regulation. You also have other rights including rights of correction, erasure, objection, and data portability. Questions, comments and requests about your personal data can also be sent to the University of Hull Information Compliance Manager Mr Luke Thompson (l.thompson3@hull.ac.uk). If you wish to lodge a complaint with the Information Commissioner's Office, please visit www.ico.org.uk.

What if I change my mind about taking part?

You are free withdraw at any point of the study, without having to give a reason. Withdrawing from the study will not affect you in any way. If you choose to withdraw from the study within 6 months after having taken part, we will delete all information you have given thus far (personal data and

research data). If you choose to withdraw after this date, we will be able to delete all your personal data (incl database entry), but not your research data. This is because after six months, your research data will already have been committed to the final report.

What will happen to the results of the study?

The results of the study will be summarised in a research article, which will be submitted for publication in an academic journal. If you are interested in obtaining a copy of this publication, please email the research team 12 months after having taken part.

Who has reviewed this study?

Research studies are reviewed by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and been given a favourable opinion by the Faculty of Health Sciences Ethics Committee, University of Hull].

Who should I contact for further information?

If you have any questions or require more information about this study, please contact me using the following contact details:

- email: h.holle@hull.ac.uk
- phone: 01482 466152

What if I have further questions, or if something goes wrong?

If you wish to make a complaint about the conduct of the study, you can contact the University of Hull using the details below for further advice and information:

In the first instance please contact Dr Henning Holle, h.holle@hull.ac.uk.

Alternatively please contact registrar@hull.ac.uk

Thank you for reading this information sheet and for considering taking part in this research.

Appendix B

INFORMATION SHEET FOR PARTICIPANTS (Paid)

YOU WILL BE GIVEN A COPY OF THIS INFORMATION SHEET

Title of study: The role of inhibitory and excitatory motor processes in planned scratch responses

I would like to invite you to participate in a research project which forms part of my ongoing research on the role of the motor system in itch. Before you decide whether you want to take part, it is important for you to understand why the research is being done and what your participation will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask me if there is anything that is not clear or if you would like more information.

What is the purpose of the study?

The purpose of the study is to study the role of the motor system when experiencing the urge to scratch. We hope to gain a better understanding of what gives rise to the intention to scratch an itch as the inhibitory processes that counteract acting out an urge to scratch.

Why have I been invited to take part?

You have been invited to participate in this study because you have indicated your interest on the SONA study information site, are healthy, and over 18 years of age.

What will happen if I take part?

A researcher will meet you to go over the information sheet and explain the procedures. The researcher will go through a Screening Form with you to make sure that it is safe for you to participate in the study. If you are happy to continue they will then ask you to sign a consent form. This study includes two visits to the TMS lab at the University of Hull. The first visit may take up to 90 minutes, the second one is shorter and is typically about 30 minutes, so the overall time commitment for this study is about 2 hours.

What is TMS?

Taking part in this study involves Transcranial Magnetic Stimulation (TMS). TMS is generally very safe, provided you meet the criteria of the medical screening. TMS is a technique that allows us to stimulate the brain by rapid switching of a magnetic field in a coil placed over the head. We can measure the effects of this stimulation by recording the activity of muscles (electromyography; EMG). EMG activity of the muscle is measured at the surface of the skin by attaching an electrode (small silver disc). Several electrodes will be taped on the skin over muscles on your hands for this purpose.

In the present study, we will use TMS to stimulate your brain using single pulse stimulation (known as single pulse TMS). Single pulses (separated by a few seconds will be applied over the scalp. At the same time, the activity in your muscles will be measuring using EMG or you may be asked to complete a task on the computer. You will be told to either contract or relax your muscles.

What do I have to do?

Before you take part in our study, we ask that you get a good night's sleep the night before, so that you are alert. Also, we ask you to refrain from excessive alcohol consumption (more than 3 units) the day before the study and any alcohol consumption on the day. We also ask that you refrain from use of recreational drugs before the study. You may drink coffee or tea as normal but we ask that you do not have a coffee for one hour before the study. If you are unsure about any of the above, please discuss these with the researcher before taking part.

Study schedule

Taking part in this study will involve an initial TMS session, to give you an idea what this form of stimulation feels like. You will receive detailed information about what taken part in the study involves, both in written and verbal form, and will be able to ask any questions you might have. We will then apply a skin prick test followed by single pulse TMS for a period of up to 10 minutes. A week later, you will be asked to come back for a second experimental session, where we will apply another skin prick test, followed by single pulse TMS for a period of up to 10 minutes.

If you choose to take part in the study, you will be asked to complete the medical screening questionnaires (one for TMS and one for the histamine prick test) and the consent form. The experimenter will be happy to answer any questions if you are not sure about any of the questions on any of these forms.

Database of regular TMS participants

If you decide to take part in this this study, we would like to invite you to become part of our pool of regular TMS participants. For this purpose, we ask on the consent form for your permission to store

your contact details, so that we can contact you to let you know about upcoming TMS experiments in the future. Becoming a member of the database does not mean that you automatically agree to take part in future studies; we will always send you all study information first so that you can make an informed choice.

If agree to become part of pool of regular participants, we will store your contact details in a safe place, for as long as you are a student at the University of Hull. After you have left the University, we will delete your contact details from the database. If you change your mind about the participant [then please let us know via email. We will then immediately delete all your contact details from the database. The following three people are conducting TMS experiments in the department and will have access to this database:

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- Dr Emmanuele Tidoni
- Dr Igor Schindler

Do I have to take part?

Participation is completely voluntary. You should only take part if you want to and choosing not to take part will not disadvantage you in any way. Once you have read the information sheet, please contact us if you have any questions that will help you make a decision about taking part. If you decide to take part, we will ask you to sign a consent form and you will be given a copy of this consent form to keep.

Payment/Incentives & benefit of taking part

You can receive a £16 Amazon voucher for participating in the study's 2 sessions.

There is no other direct benefit for you in taking part in this study. Your results will however help to gain scientific knowledge on the neural processes underpinning acute itch.

What are the possible risks of taking part?

TMS-specific risks

TMS can sometimes cause a mild headache or muscle spasms, which invariably settle with time or with simple analgesics (common pain medication, such as paracetamol). Experimenters will observe you and question you to check that you are comfortable during the experiment. Prolonged, high intensity, high frequency TMS has very rarely been reported to induce brief seizures (less than 1 in 1000 studies). In most cases, the seizure was associated with a family history of epilepsy, existing neurological disease (e.g. multiple sclerosis) or medication (anti-depressant or dopamine medication). The risk of a provoked seizure occurring in healthy individuals due to TMS is extremely

small. As a precaution, you cannot take part in TMS if you have a personal or close family history of epilepsy. If you are taking any medication, you should discuss this with the researcher beforehand. If you suffer with migraine headaches, you should not take part in this study.

Risks associated with the histamine prick test

Taking part in the study will involve the experience of itch. The itch experienced after a histamine prick is similar to mosquito bite, although less intense. Itch and associated reddening of the skin have usually completely subsided 30 to 60 minutes after the application. The histamine prick test usually does not lead to bleeding. At most, there may be a droplet of blood. The experimenter has been trained in the correct application of the prick test.

When larger doses of histamine are infused directly into the blood stream, a number of unwanted side effects can occur. This side effects include headache, developing an itchy rash (urticaria), drop in blood pressure, constriction of the airways (bronchospasm) and cramp-like abdominal pains. In the present study, only a very small amount of histamine solution will be deposited in the upper skin layers of the test site (up to 2 µl, which is equivalent to 0.002 ml). Most of this small amount of histamine solution will remain at the site of skin prick, and very little (if any) will enter your blood stream. It is therefore extremely unlikely that you will experience any of the above-mentioned side effects. Nonetheless, we have to make you aware that these side effects exist.

In order to make sure that it is safe for you to take part, you have to answer a number of questions about your medical history and any medication you are currently receiving (please see the histamine prick test screening form for details).

In short, the test area, which is located in the middle of your forearm (see Figure on consent form), should be free from

- skin infections

- acute or chronic eczema

- signs of increased skin reactivity. Examples of very sensitive skin include skin that allows ‘skin writing’, or abnormally thick, dry or scaly skin

You also shouldn’t be hypersensitive to any of the ingredients of the histamine solution, which are (apart from water and salt)

- Histamine
- Phenol
- Glycerol

- Sodium Hydroxide

You also shouldn't

- currently suffer from acute allergic symptoms
- suffer from a serious general disorder
- currently have a fever
- receive treatment with β-Blockers
- suffer from any disease of the heart or blood vessels (cardiovascular disease)
- have a history of low blood pressure
- have a history of fainting during medical procedures (e.g., during a flu shot, or immunization shot)
- suffer from asthma
- be pregnant or breastfeeding
- have taken antihistamines in the last 48 hours

Some people suffer from a condition called histamine intolerance. When eating histamine-rich foods (e.g., spinach, sauerkraut, certain types of sausage and fish), histamine-intolerant people tend to develop 'allergy-like' symptoms such as headaches, rashes, itching, diarrhoea, and vomiting or abdominal pain. If you suffer from histamine intolerance, you should not take part in this experiment (as a precautionary measure). If a histamine-intolerant person undergoes a histamine prick test, it may take longer than 30 – 60 minutes until the reddening of the skin has completely subsided.

No other risks of the histamine prick test are known to the investigator at this time.

Data handling and confidentiality

In this research study we will use information from you. In particular, this will be your personal data (name and contact details, and your individual brain scan) and the research data (the reaction time data). Your data will be processed in accordance with the General Data Protection Regulation 2016 (GDPR). We will only use information that we need for the research study. We will let very few people know your name or contact details, and only if they really need it for this study.

Everyone involved in this study will keep your data safe and secure. We will also follow all privacy rules. We will make sure noone can work out who you are from the reports we write.

With your agreement, your personal data (name and email address) will be stored in a secure database. In this database, we will also add a random identification code next to your name. All your other data (the recorded EMGs) will only contain that random identification code, but not your real name or other information that could identify you. Thus, in the unlikely event that an external person should get access to the research data, they will not be able to identify you, since these data have been anonymized.

The only people who will have access to the database with your contact details will be the Primary Investigator of this study, Dr Henning Holle, as well as Dr Igor Schindler and Dr Emmanuele Tidoni from the Department of Psychology. Your personal data will not be shared with any third parties. Your research data will be used to support current and future research and may be shared anonymously with other researchers.

We will store the anonymized research data for a period of 10 years. We will store the written consent forms for a period of 6 months. We will store your contact details for as long as you are a student of the University of Hull.

Data Protection Statement

The data controller for this project will be the University of Hull. The University will process your personal data for the purpose of the research outlined above. The legal basis for processing your personal data for research purposes under GDPR is a 'task in the public interest' You can provide your consent for the use of your personal data in this study by completing the consent form that has been provided to you. Information about how the University of Hull processes your data can be found at https://www.hull.ac.uk/choose-hull/university-and-region/key-documents/data-protection.aspx

You have the right to access information held about you. Your right of access can be exercised in accordance with the General Data Protection Regulation. You also have other rights including rights of correction, erasure, objection, and data portability. Questions, comments and requests about your personal data can also be sent to the University of Hull Information Compliance Manager Mr Luke Thompson (l.thompson3@hull.ac.uk). If you wish to lodge a complaint with the Information Commissioner's Office, please visit www.ico.org.uk.

What if I change my mind about taking part?

You are free withdraw at any point of the study, without having to give a reason. Withdrawing from the study will not affect you in any way. If you choose to withdraw from the study within 6 months after having taken part, we will delete all information you have given thus far (personal data and

research data). If you choose to withdraw after this date, we will be able to delete all your personal data (incl database entry), but not your research data. This is because after six months, your research data will already have been committed to the final report.

What will happen to the results of the study?

The results of the study will be summarised in a research article, which will be submitted for publication in an academic journal. If you are interested in obtaining a copy of this publication, please email the research team 12 months after having taken part.

Who has reviewed this study?

Research studies are reviewed by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and been given a favourable opinion by the Faculty of Health Sciences Ethics Committee, University of Hull].

Who should I contact for further information?

If you have any questions or require more information about this study, please contact me using the following contact details:

- email: h.holle@hull.ac.uk
- phone: 01482 466152

What if I have further questions, or if something goes wrong?

If you wish to make a complaint about the conduct of the study, you can contact the University of Hull using the details below for further advice and information:

In the first instance please contact Dr Henning Holle, h.holle@hull.ac.uk.

Alternatively please contact registrar@hull.ac.uk

Thank you for reading this information sheet and for considering taking part in this research.

Appendix C

Version number and date: 1.0, 13 September 2019

CONSENT FORM

Title of study: The role of inhibitory and excitatory motor processes in planned scratch responses

Name of Researcher: Dr Henning Holle

	Please initial box
1.I confirm that I have read the information sheet dated..**13 September 2019**. version. ... **1.0..** for the above study. I have had the opportunity to consider the information, ask questions and have had any questions answered satisfactorily.	
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, and without my legal rights being affected. I understand that after a period of 6 months, I can no longer withdraw my anonymised research data.	
3. I understand that the research data, which will be anonymised (not linked to me), will be retained by the researchers and may be shared with others and publicly disseminated to support other research in the future.	
4. I agree to be entered in the pool of regular TMS participants. For this purpose, I give permission that my personal details (name and email adress) are stored in a secure database. I understand that my personal data will be kept securely in accordance with data protection guidelines, and will only be available to Primary Investigator of this study, Dr Henning Holle, as well as Dr Igor Schindler and Dr Emmanuele Tidoni. I understand that my personal details will be stored for as long as I am a student at the University of Hull and will be deleted once I have left the University Please provide your University of Hull email adress for this purpose:	
5. I understand that I can withdraw from the pool of regular TMS participants at any time	
6.I agree to take part in the above study.	

Name of Participant	Date	Signature
Name of Person taking consent	Date	Signature

Appendix D

The information you provide is for screening purposes only and will be kept completely confidential.

(1) Do you have epilepsy or have you ever had a convulsion or a seizure? YES/NO

(2) Does anyone in your immediate or distant family suffer from epilepsy? YES/NO

(3) Have you ever had a fainting spell or syncope? If yes, please describe on which occasion(s) YES/NO

(4) Have you ever had a head trauma that was diagnosed as a concussion or was associated with loss of consciousness? YES/NO

(5) Do you have any hearing problems or ringing in your ears? YES/NO

(6) Do you have cochlear implants? YES/NO

(7) Are you pregnant or is there any chance that you might be? YES/NO

(8) Do you have metal in the brain, skull or elsewhere in your body (e.g., splinters, fragments, clips, etc.)? YES/NO

(9) Do you have an implanted neurostimulator (e.g., DBS, epidural/subdural, VNS)? YES/NO

(10) Do you have a cardiac pacemaker or intracardiac lines? YES/NO

(11) Do you have a medication infusion device? YES/NO

(12) Are you taking any medications or are currently being treated for a mental disorder? YES/NO

(please list)

(13) Have you consumed alcohol or drugs within the last 24 hours? YES/NO

(14) Have you slept an unusual small amount of hours? YES/NO

(15) Did you ever undergo TMS in the past? YES/NO

If so, were there any problems. YES/NO

(17) Do you currently have tooth ache? YES/NO

(18) Are you currently taking any unprescribed medication? YES/NO

If yes, please give details.

(19) Can you think of any other reason(s) than the ones stated for you not to take part in the study?

YES/NO

If yes, please give details.

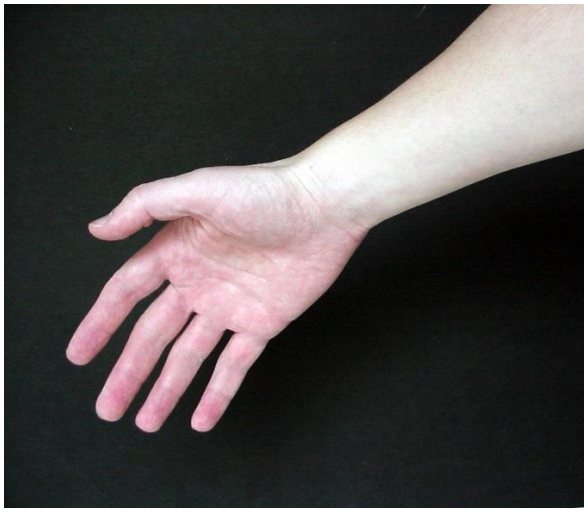
I (please give full name in CAPITALS) ....., confirm that I have read the letter of invitation and have completed the above questionnaire. The nature and possible consequences of the procedures involved have been explained to me. I understand that I may withdraw from the study at any time.

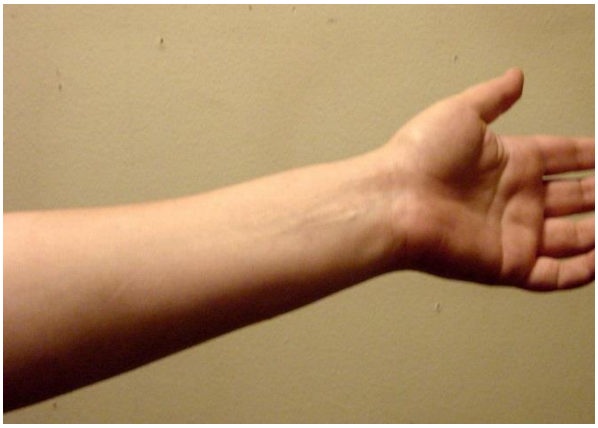
Signature & Date:

Please note: All data arising from this study will be held and used in accordance with the Data Protection Act (2018). The results of the study will not be made available in a way which could reveal the identity of individuals.

The information you provide is for screening purposes only and will be kept completely confidential.

If female, are you currently pregnant or breastfeeding? YES/NO





Do you have any of the following in the test area: left inner arm or right inner arm (**see circled area above**)

- Wounds, rashes, swelling or reddening YES/NO
- Tattoos YES/NO
- Scars YES/NO
- Creams which you have applied in the past 24 hours (e.g. moisturizer) YES/NO
- Very sensitive skin (e.g., skin that allows skin writing, dry, thick or scaly skin) YES/NO

Have you taken any of the medication/drugs **in the past 48 hours**:

- Antihistamines (e.g., as a treatment for hayfever) YES/NO
- Beta blockers (e.g., for treatment of heart condition) YES/NO

Are you currently taking **any** medication regularly, other than oral contraceptive,

i.e., 'the pill' YES/NO

If yes, please list all medication(s) you are currently taking on a regular basis

Do you currently suffer from or have a history of any of the following:

- Fainting during medical procedures (e.g., flu shots or immunization shots) YES/NO
- An allergy? YES/NO
- An acute or chronic skin condition (e.g. eczema, psoriasis)? YES/NO
- Any disease of the heart or blood vessels (cardiovascular disease)? YES/NO
- Low blood pressure? YES/NO
- Fever? YES/NO
- Asthma? YES/NO
- Histamine Intolerance YES/NO

Are you hypersensitive to any of the following substances?

- Histamine (spinach, sauerkraut, certain types of sausage and cheese are rich in histamine) YES/NO
- Phenol (many types of berries and fruit are rich in phenol) YES/NO
- Glycerol (milk, clotted cream, puddings and yogurt are rich in glycerol) YES/NO
- Sodium Hydroxide (a.k.a. lye or caustic soda, used for preparation of pretzels, chinese noodles, also used in production of soft drinks) YES/NO

I (please give full name in CAPITALS)..... , confirm that I have read the letter of invitation and have completed the above questionnaire. The nature and possible consequences of the procedures involved have been explained to me. I understand that I may withdraw from the study at any time.

Signature & Date:

Please note: All data arising from this study will be held and used in accordance with the Data Protection Act (2018). The results of the study will not be made available in a way which could reveal the identity of individuals.

Appendix E

Edinburgh Handedness Inventory

Surname_____Given Name_____

Date of

Birth_____Sex_____

Please indicate your preferences in the use of hands in the following activities by *putting + in the appropriate column*. Where the preference is so strong that you would never try to use the other hand unless absolutely forces to, *put ++*. If any case you are really indifferent put + in both columns.

Some of the activities require both hands. In these cases the part of the task, or object, for which hand preference is wanted is indicated in brackets.

Please try to answer all the questions, and only leave a blank if you have no experience at all of the object or task.

	Left	Right
1. Writing		
2. Drawing		
3. Throwing		
4. Scissors		
5. Toothbrush		
6. Knife (without fork)		
7. Spoon		
8. Broom (upper hand)		
9. Striking Match (match)		
10. Opening box (lid)		
i. Which foot do you prefer to kick with?		
ii. Which eye do you use when using only one?		

L.Q.	Leave the spaces blank	DECLE

Appendix F

Debriefing Information

Title: The Effect of Acute Itch On Motor System Excitability (Contralateral)

Name of Principal Investigator and Researcher – Dr Henning Holle and Matthew Page

Background

Itch is an unpleasant skin sensation that triggers the urge to scratch. Histamine is the most common mediator of itch, thus, it was used in the research to induce itch, when you received a skin prick per session. Regarding actual scratching, the behaviour serves as a pleasurable mild pain to relieve itchiness (Paus et al, 2006). The aim of this study is to investigate the role of motor cortical excitation which gives rise to the urge to scratch. Excitation was measured through motor evoked potentials (MEP) elicited by transcranial magnetic stimulation over the primary motor cortex. An MEP is a brief muscle response from brain stimulation over the motor system, recorded with an EMG. Past research measuring MEPs during muscle pain showed contralateral motor cortex inhibition to the side of pain (Pera et al, 2001) (Burns et al, 2016), but no research currently shows the pattern of MEPs during itch.

Anticipated Findings

Based on Pera et al (2001) and Burns et al (2016), it was anticipated a similar outcome will occur, that MEPs evoked by TMS will show contralateral motor cortical inhibition to the side of itch.

Further Information

If you have any complaints, concerns or questions about this research, please feel free to contact Dr.Henning Holle (h.holle@hull.ac.uk) or Matthew Page (m.l.page-2015@hull.ac.uk).

Appendix G

Debriefing Information

Title: The Effect of Acute Itch on Motor System Excitability (Ipsilateral)

Name of Principal Investigator and Researcher – Dr Henning Holle and Matthew Page

Background

Itch is an unpleasant skin sensation that triggers the urge to scratch. Histamine is the most common mediator of itch, thus, it was used in the research to induce itch, when you received a skin prick per session. Regarding actual scratching, the behaviour serves as a pleasurable mild pain to relieve itchiness (Paus et al, 2006). The aim of this study is to investigate the role of motor cortical excitation which gives rise to the urge to scratch. Excitation was measured through motor evoked potentials (MEP) elicited by transcranial magnetic stimulation over the primary motor cortex. An MEP is a brief muscle response from brain stimulation over the motor system, recorded with an EMG. Past research using brain imaging data showed that the motor cortex was activated during itch sensations, so it is hypothesised that this region is responsible for the urge to scratch. However, no research currently shows the pattern of MEPs during itch.

Anticipated Findings

It is hypothesised that MEP amplitudes will increase during an itch sensation period compared to a baseline or placebo.

Further Information

If you have any complaints, concerns or questions about this research, please feel free to contact Dr.Henning Holle (h.holle@hull.ac.uk) or Matthew Page (m.l.page-2015@hull.ac.uk).

Appendix H

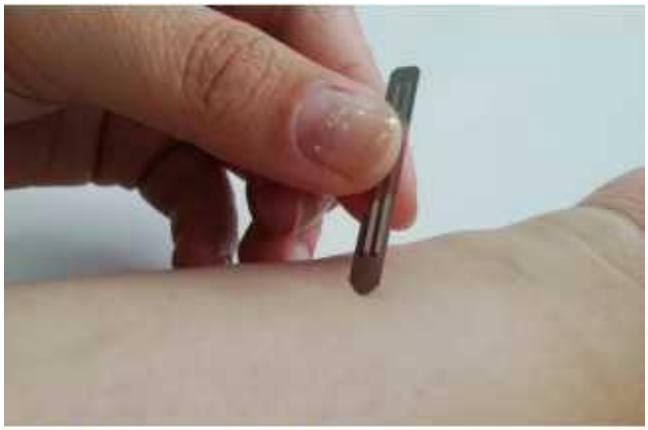


Figure 1 The Histamine Prick Test

Appendix I

```
NPTESTS
  /ONESAMPLE TEST (PCT_Diff_Placebo_first PCT_Diff_Placebo_Second PCT_Diff_Histamine_First
    PCT_Diff_Histamine_Second) WILCOXON(TESTVALUE=100)
  /MISSING SCOPE=ANALYSIS USERMISSING=EXCLUDE
  /CRITERIA ALPHA=0.05 CILEVEL=95  SEED=2000000.
```

Nonparametric Tests_Study 1_ipsi

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.[a,b]
1	The median of PCT_Diff_Placebo_first equals 100.00.	One-Sample Wilcoxon Signed Rank Test	.796
2	The median of PCT_Diff_Placebo_Second equals 100.00.	One-Sample Wilcoxon Signed Rank Test	.034
3	The median of PCT_Diff_Histamine_First equals 100.00.	One-Sample Wilcoxon Signed Rank Test	.179
4	The median of PCT_Diff_Histamine_Second equals 100.00.	One-Sample Wilcoxon Signed Rank Test	.001

Hypothesis Test Summary

	Decision
1	Retain the null hypothesis.
2	Reject the null hypothesis.
3	Retain the null hypothesis.

4	Reject the null hypothesis.

a. The significance level is .050.

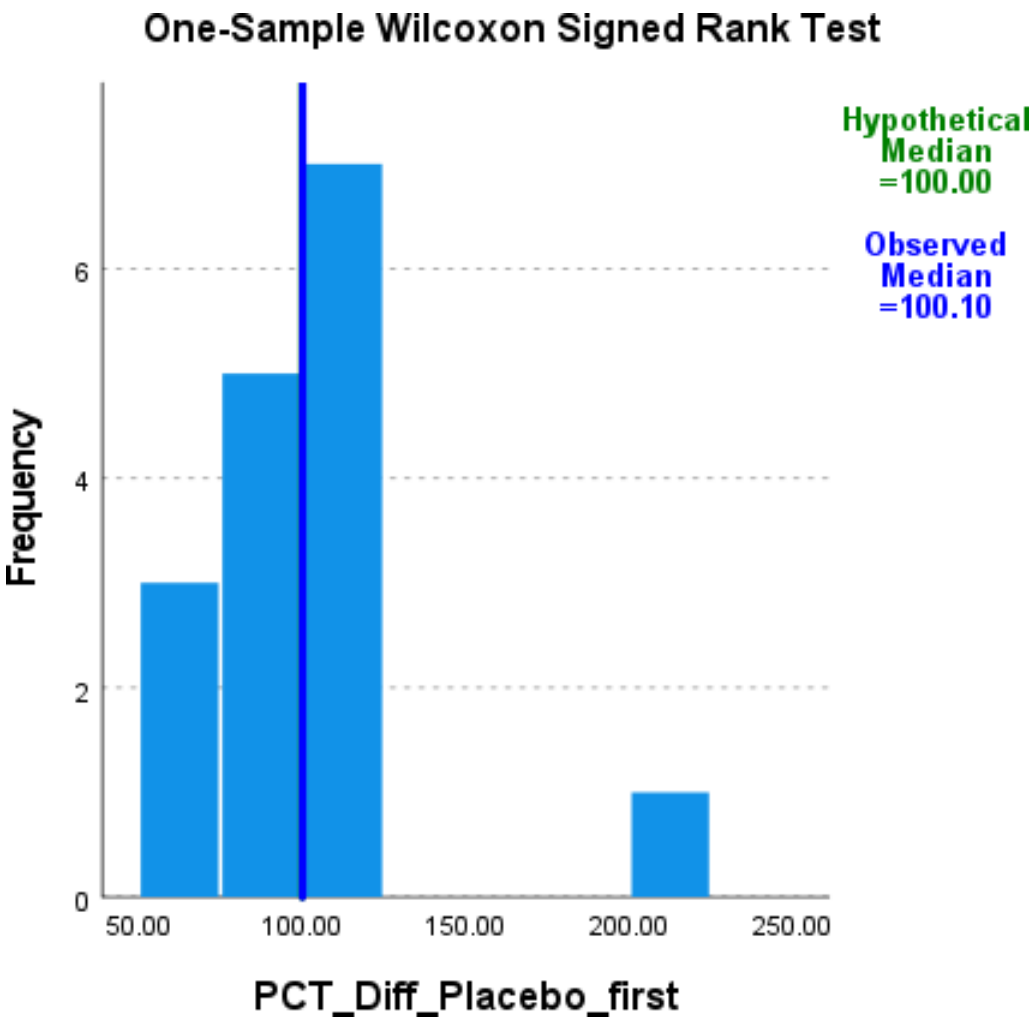
b. Asymptotic significance is displayed.

One-Sample Wilcoxon Signed Rank Test

PCT_Diff_Placebo_first

One-Sample Wilcoxon Signed Rank Test Summary

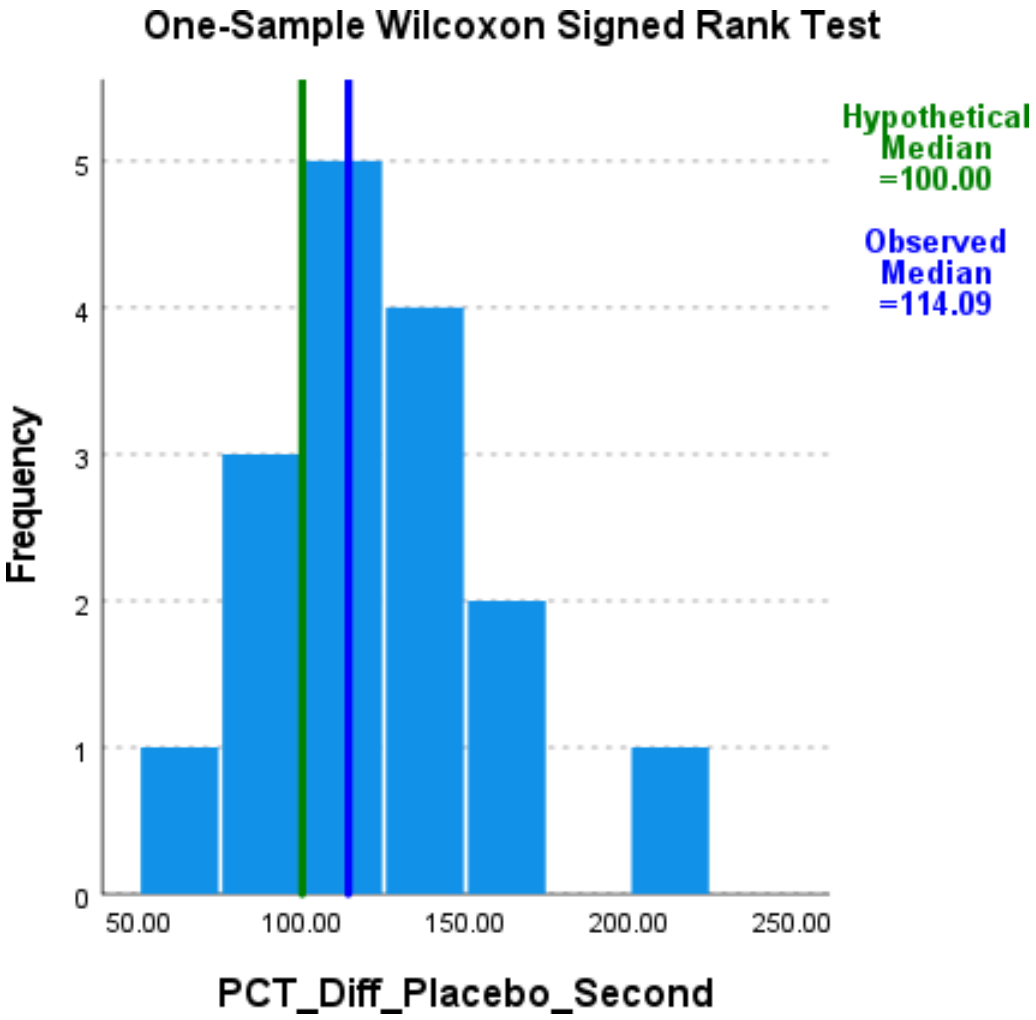
Total N	16
Test Statistic	63.000
Standard Error	19.339
Standardized Test Statistic	-.259
Asymptotic Sig.(2-sided test)	.796


One-Sample Wilcoxon Signed Rank Test
Hypothetical Median =100.00
Observed Median =100.10
Frequency
0
2
4
6
50.00
100.00
150.00
200.00
250.00
PCT_Diff_Placebo_first

PCT_Diff_Placebo_Second

One-Sample Wilcoxon Signed Rank Test Summary

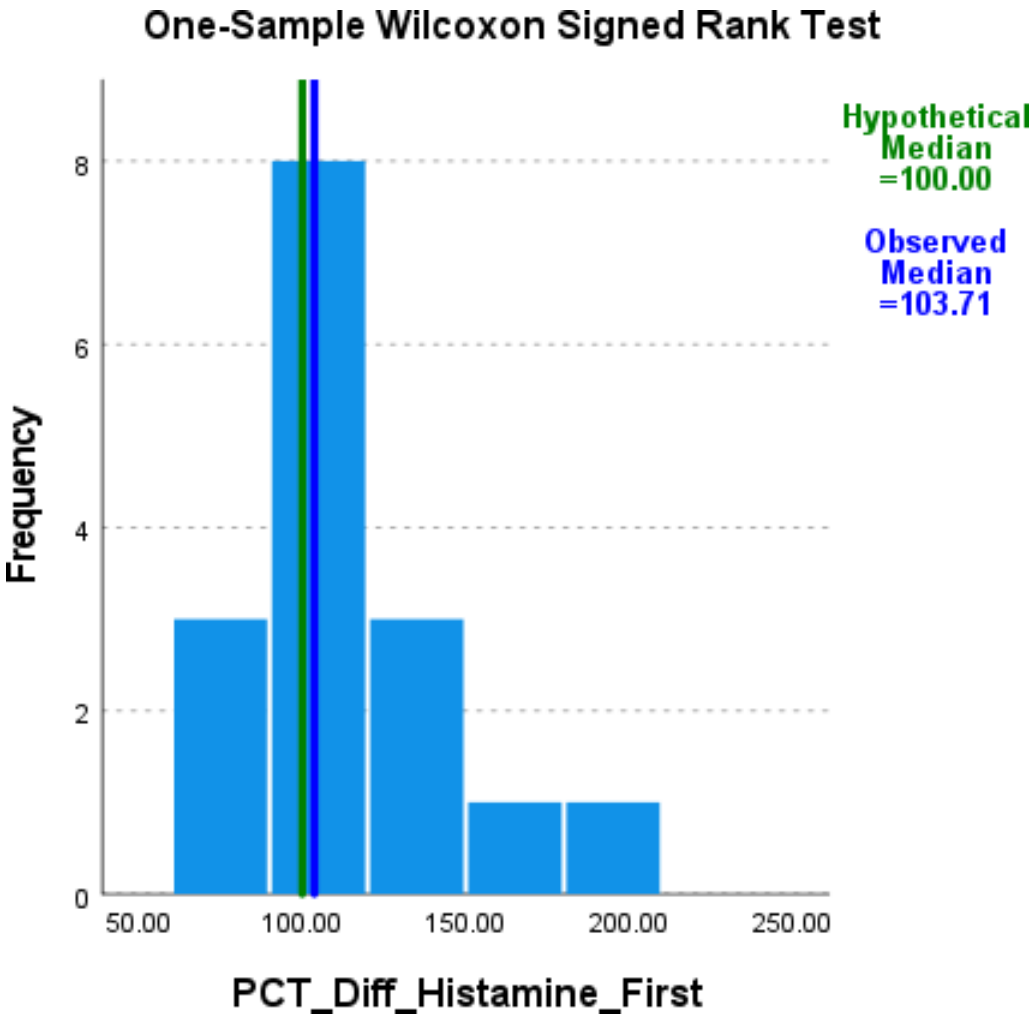
Total N	16
Test Statistic	109.000
Standard Error	19.339
Standardized Test Statistic	2.120
Asymptotic Sig.(2-sided test)	.034


One-Sample Wilcoxon Signed Rank Test
Hypothetical Median =100.00
Observed Median =114.09
Frequency
0
1
2
3
4
5
50.00
100.00
150.00
200.00
250.00
PCT_Diff_Placebo_Second

PCT_Diff_Histamine_First

One-Sample Wilcoxon Signed Rank Test Summary

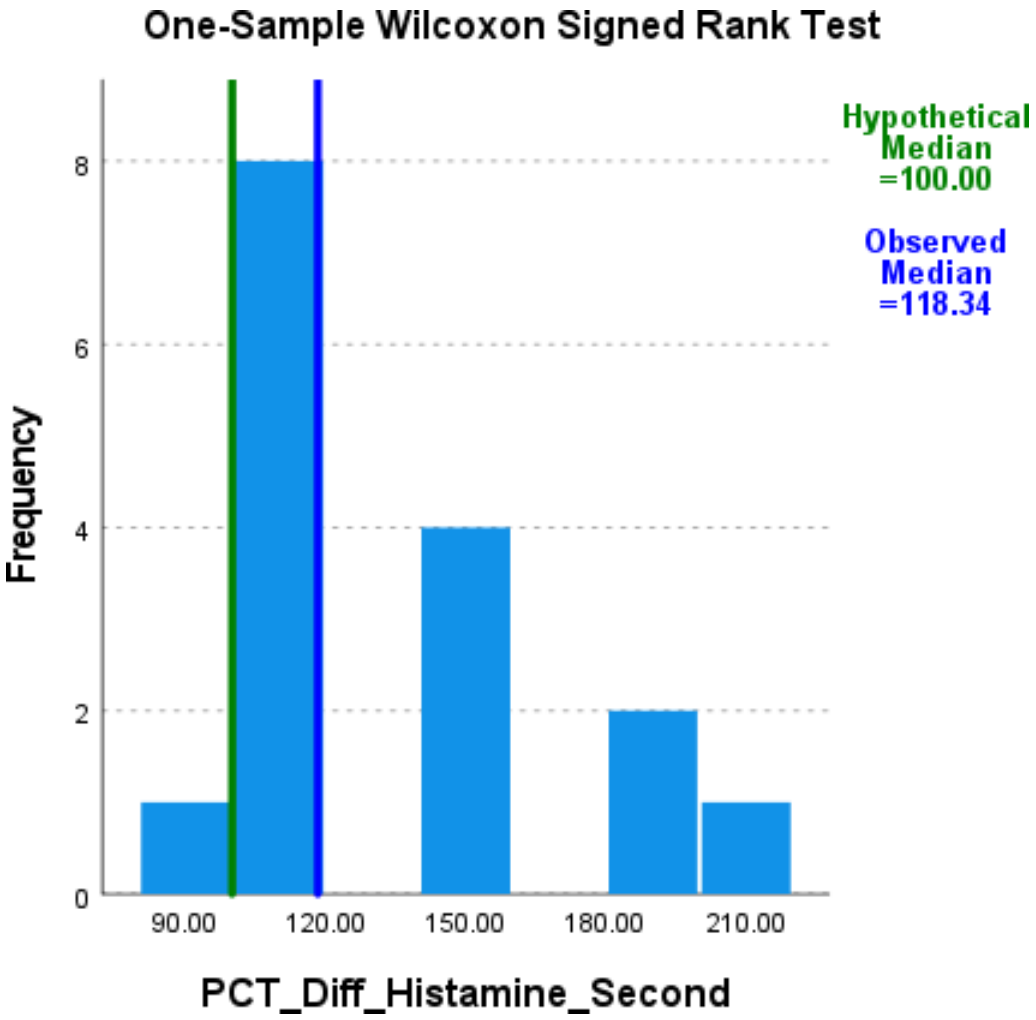
Total N	16
Test Statistic	94.000
Standard Error	19.336
Standardized Test Statistic	1.345
Asymptotic Sig.(2-sided test)	.179

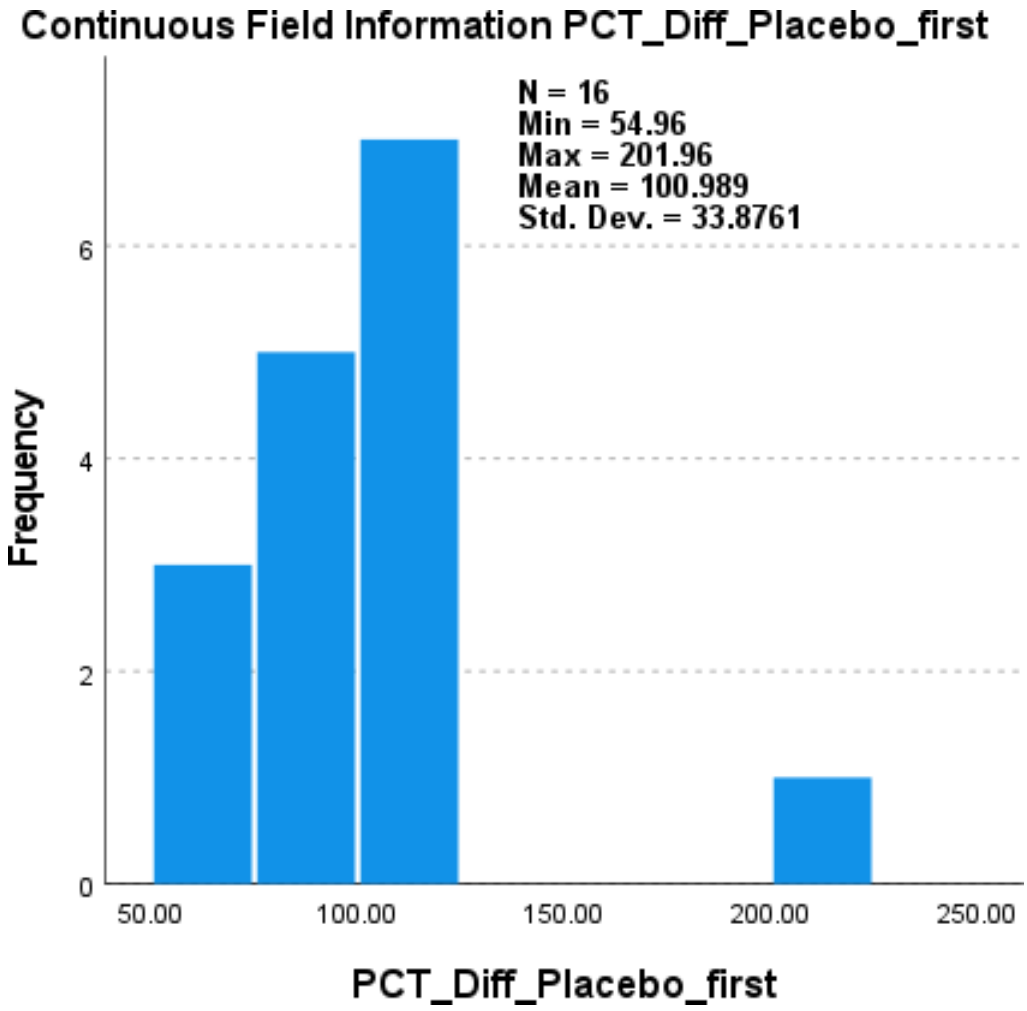

One-Sample Wilcoxon Signed Rank Test
Hypothetical Median =100.00
Observed Median =103.71
Frequency
0
2
4
6
8
50.00
100.00
150.00
200.00
250.00
PCT_Diff_Histamine_First

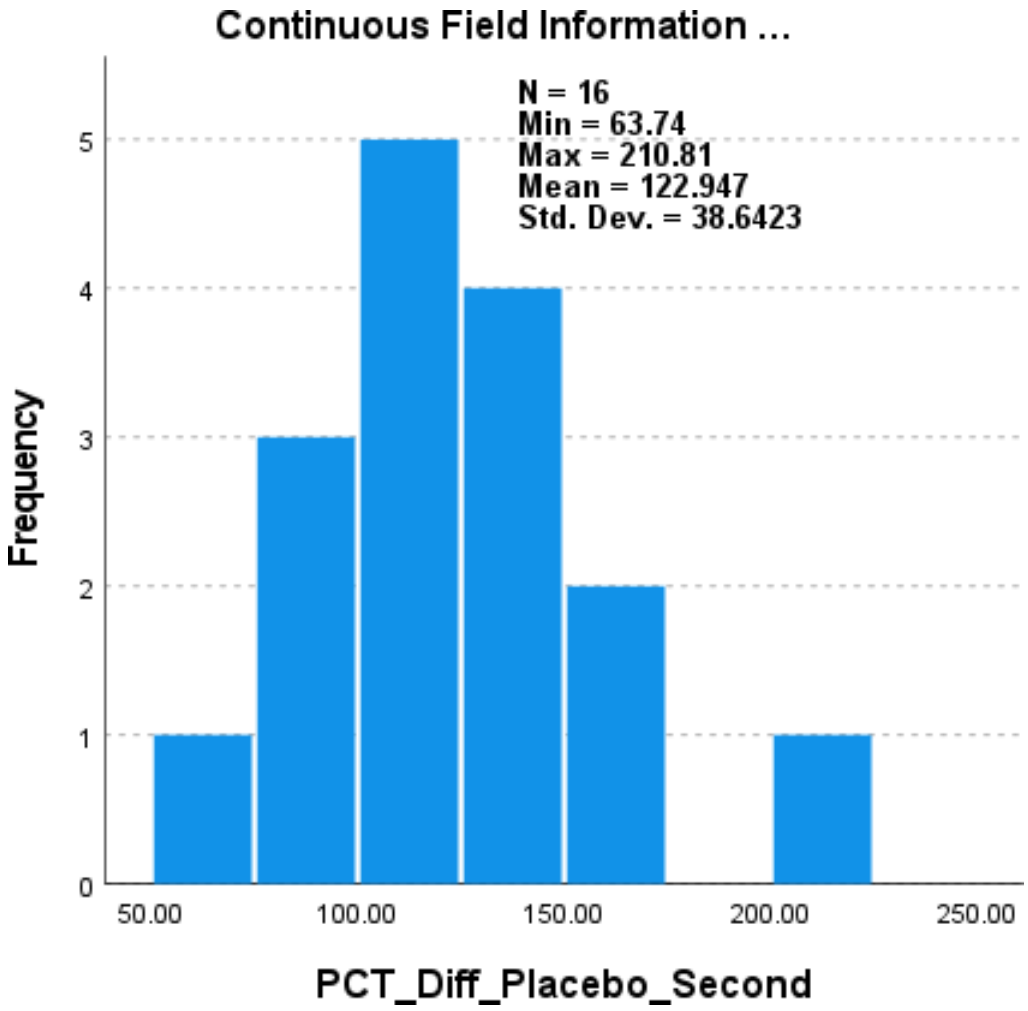
PCT_Diff_Histamine_Second

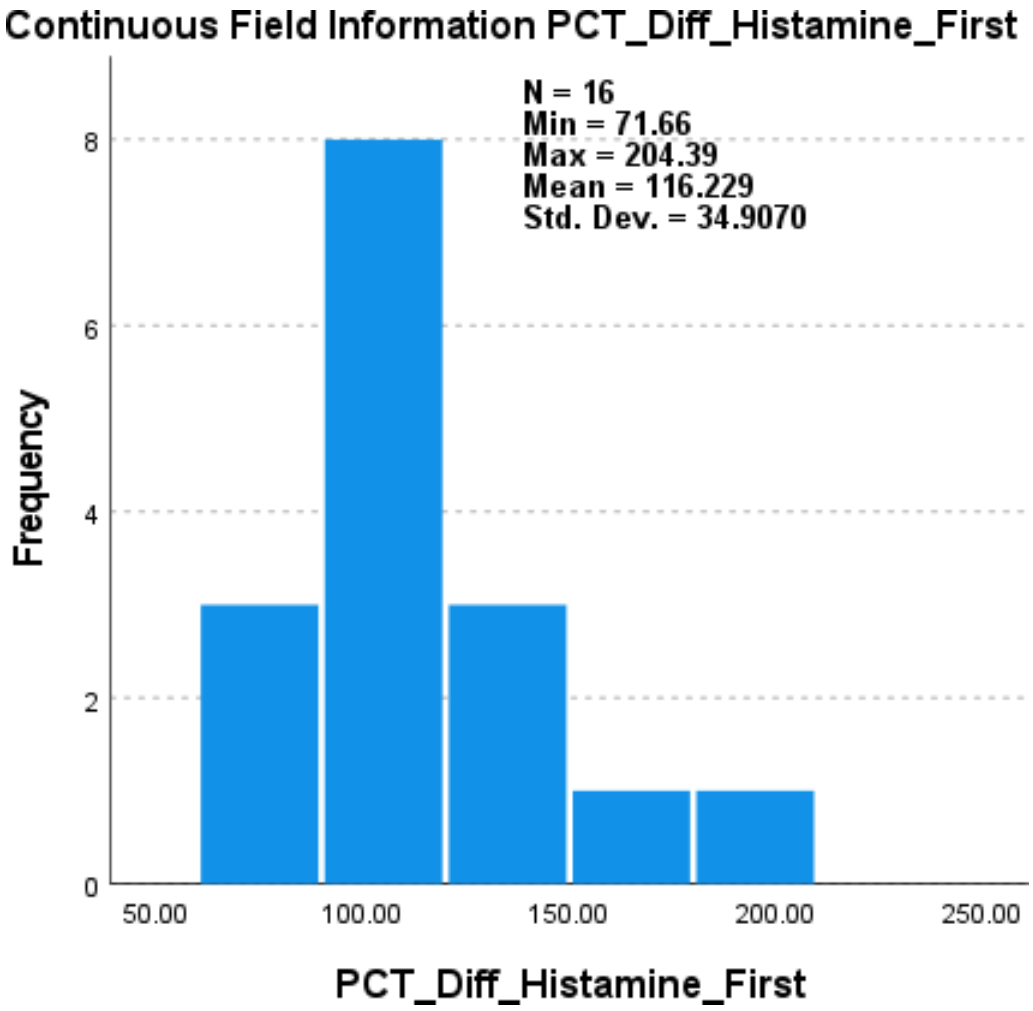
One-Sample Wilcoxon Signed Rank Test Summary

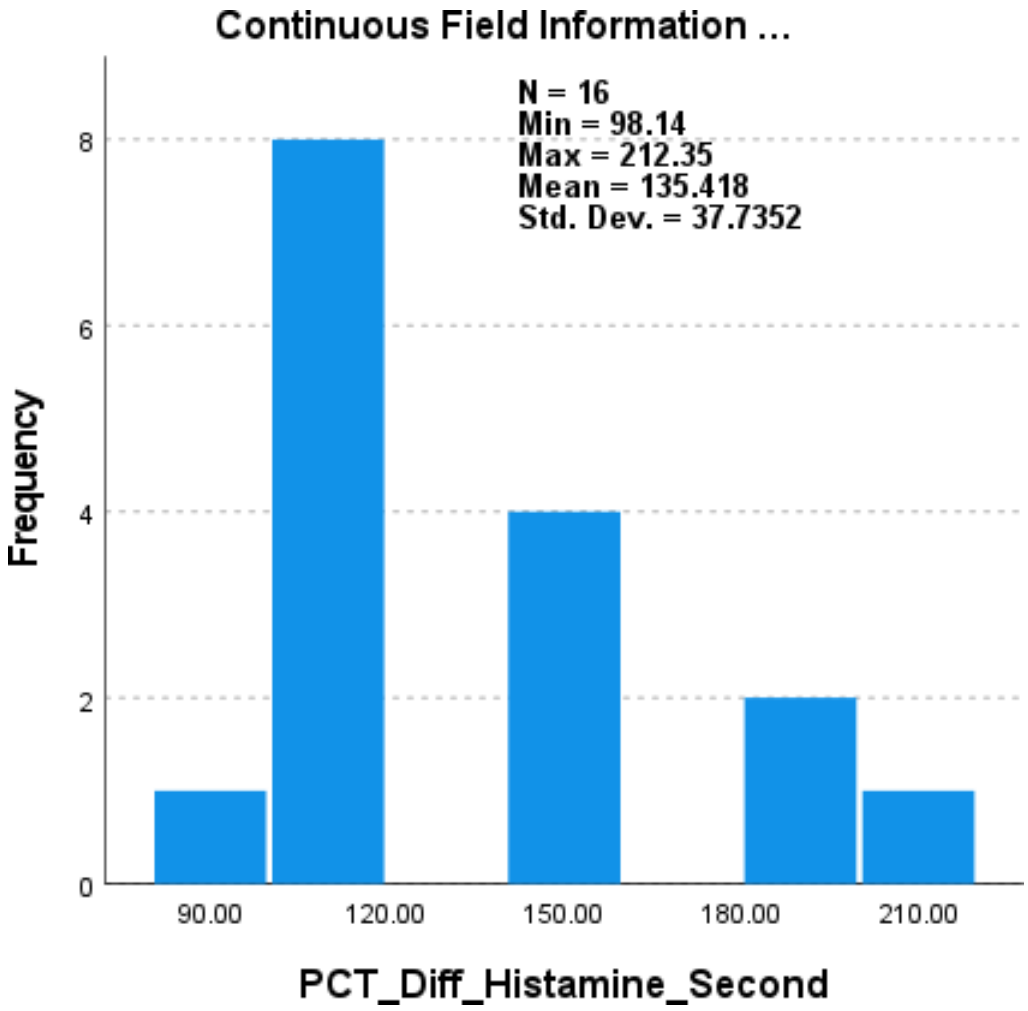
Total N	16
Test Statistic	134.000
Standard Error	19.336
Standardized Test Statistic	3.413
Asymptotic Sig.(2-sided test)	.001


One-Sample Wilcoxon Signed Rank Test
Hypothetical Median =100.00
Observed Median =118.34
Frequency
0
2
4
6
8
90.00
120.00
150.00
180.00
210.00
PCT_Diff_Histamine_Second


Continuous Field Information PCT_Diff_Placebo_first
N = 16
Min = 54.96
Max = 201.96
Mean = 100.989
Std. Dev. = 33.8761
Frequency
0
2
4
6
50.00
100.00
150.00
200.00
250.00
PCT_Diff_Placebo_first


Continuous Field Information ...
N = 16
Min = 63.74
Max = 210.81
Mean = 122.947
Std. Dev. = 38.6423
Frequency
0
1
2
3
4
5
50.00
100.00
150.00
200.00
250.00
PCT_Diff_Placebo_Second


Continuous Field Information PCT_Diff_Histamine_First
N = 16
Min = 71.66
Max = 204.39
Mean = 116.229
Std. Dev. = 34.9070
Frequency
0
2
4
6
8
50.00
100.00
150.00
200.00
250.00
PCT_Diff_Histamine_First



NPAR TESTS

/WILCOXON=H_halves_mean first_half_mean WITH P_halves_mean second_half_mean (PAIRED)

/MISSING ANALYSIS.

NPar Tests

Wilcoxon Signed Ranks Test

Ranks

		N	Mean Rank	Sum of Ranks
P_halves_mean - H_halves_mean	Negative Ranks	13[a]	8.15	106.00
	Positive Ranks	3[b]	10.00	30.00
	Ties	0[c]		
	Total	16		
second_half_mean - first_half_mean	Negative Ranks	2[d]	4.00	8.00
	Positive Ranks	14[e]	9.14	128.00
	Ties	0[f]		
	Total	16		

a. P_halves_mean < H_halves_mean

b. P_halves_mean > H_halves_mean

c. P_halves_mean = H_halves_mean

d. second_half_mean < first_half_mean

e. second_half_mean > first_half_mean

f. second_half_mean = first_half_mean

Test Statistics[a]

	P_halves_mean - H_halves_mean	second_half_mean - first_half_mean
Z	-1.965[b]	-3.103[c]
Asymp. Sig. (2-tailed)	.049	.002

a. Wilcoxon Signed Ranks Test

b. Based on positive ranks.

c. Based on negative ranks.

```
NPTESTS
 /ONESAMPLE TEST (interaction) WILCOXON(TESTVALUE=0)
 /MISSING SCOPE=ANALYSIS USERMISSING=EXCLUDE
 /CRITERIA ALPHA=0.05 CILEVEL=95  SEED=2000000.
```

Nonparametric Tests

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.[a,b]
1	The median of interaction equals .00.	One-Sample Wilcoxon Signed Rank Test	.959

Hypothesis Test Summary

	Decision
1	Retain the null hypothesis.

a. The significance level is .050.

b. Asymptotic significance is displayed.

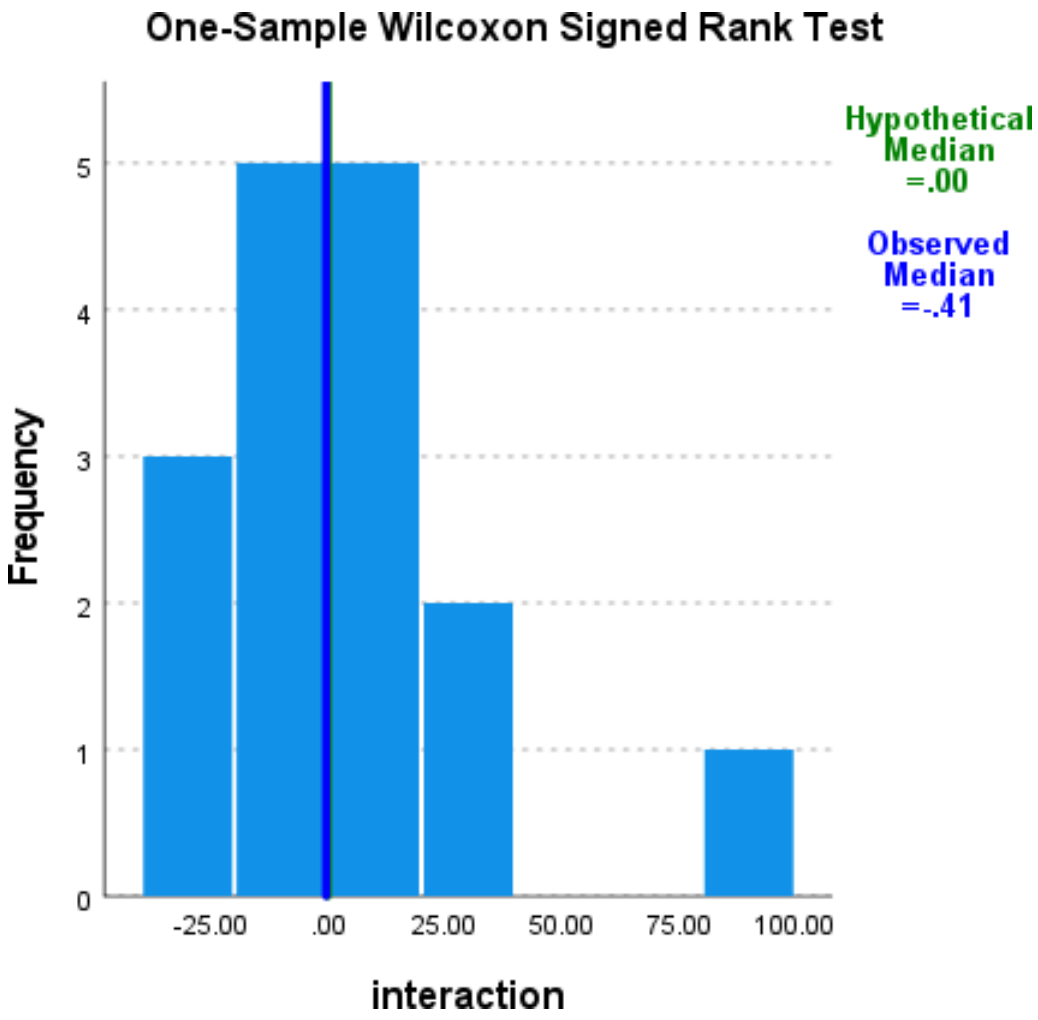
One-Sample Wilcoxon Signed Rank Test

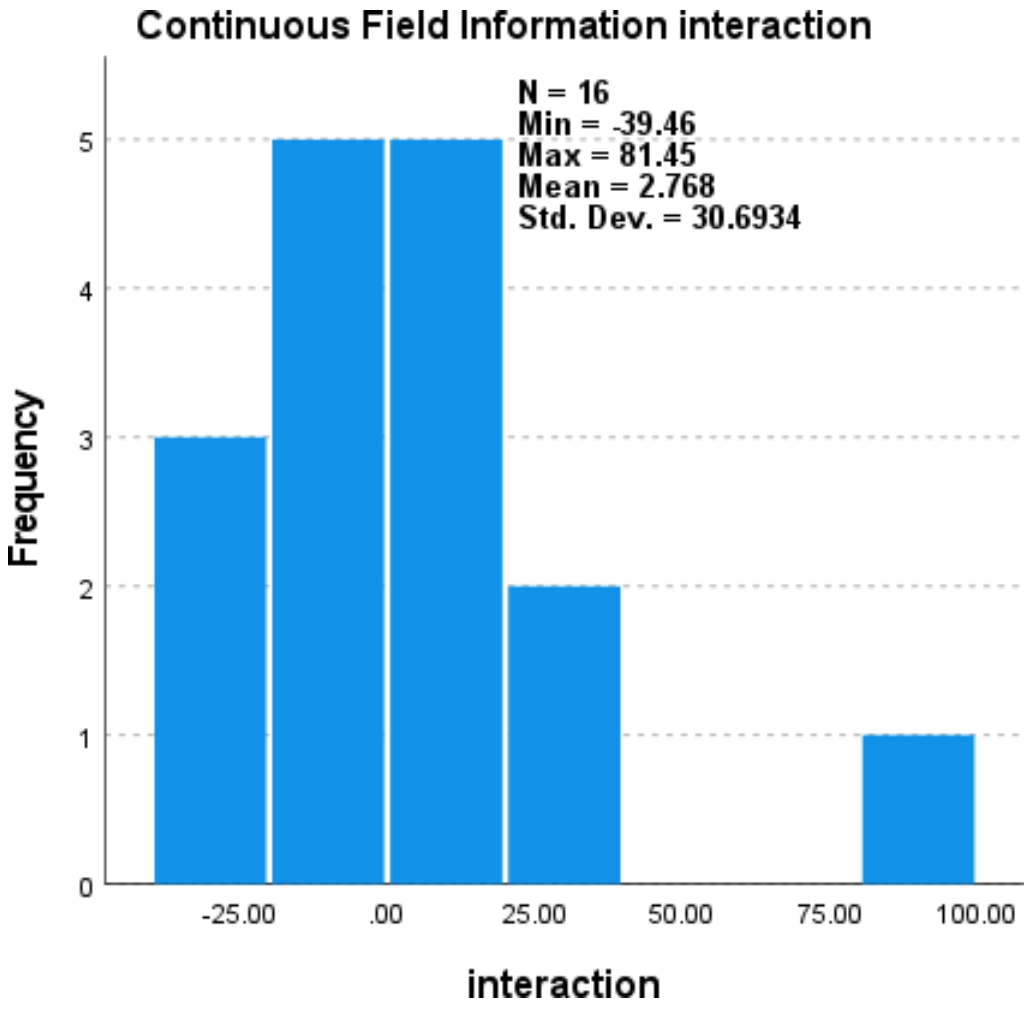
interaction

One-Sample Wilcoxon Signed Rank Test Summary

Total N	16
Test Statistic	69.000
Standard Error	19.339

Standardized Test Statistic	.052
Asymptotic Sig.(2-sided test)	.959





One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
PCT_Diff_Placebo_first	16	100.9895	33.87614	8.46903
PCT_Diff_Placebo_Second	16	122.9469	38.64227	9.66057

PCT_Diff_Histamine_First	16	116.2289	34.90699	8.72675
PCT_Diff_Histamine_Second	16	135.4181	37.73523	9.43381

Statistics

	N				
	Valid	Missing	Mean	Std. Error of Mean	Median
Placebo First Half	16	0	100.9895	8.46903	100.0961
Placebo Second Half	16	0	122.9469	9.66057	114.0876
PCT_Diff_Histamine_First	16	0	116.2289	8.72675	103.7091
PCT_Diff_Histamine_Second	16	0	135.4181	9.43381	118.3389

Statistics

	Std. Deviation	Sum
Placebo First Half	33.87614	1615.83
Placebo Second Half	38.64227	1967.15
PCT_Diff_Histamine_First	34.90699	1859.66
PCT_Diff_Histamine_Second	37.73523	2166.69

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
PCT_Diff_Placebo_first	16	100.0%	0	0.0%	16	100.0%
PCT_Diff_Placebo_Second	16	100.0%	0	0.0%	16	100.0%
PCT_Diff_Histamine_First	16	100.0%	0	0.0%	16	100.0%
PCT_Diff_Histamine_Second	16	100.0%	0	0.0%	16	100.0%

Descriptives

			Statistic	Std. Error
PCT_Diff_Placebo_first	Mean		100.9895	8.46903
	95% Confidence Interval for Mean	Lower Bound	82.9382	
		Upper Bound	119.0408	
	5% Trimmed Mean		97.9375	
	Median		100.0961	
	Variance		1147.593	
	Std. Deviation		33.87614	
	Minimum		54.96	
	Maximum		201.96	
	Range		147.00	

	Interquartile Range		35.20	
	Skewness		1.667	.564
	Kurtosis		4.824	1.091
PCT_Diff_Placebo_Second	Mean		122.9469	9.66057
	95% Confidence Interval for Mean	Lower Bound	102.3558	
		Upper Bound	143.5379	
	5% Trimmed Mean		121.3552	
	Median		114.0876	
	Variance		1493.225	
	Std. Deviation		38.64227	
	Minimum		63.74	
	Maximum		210.81	
	Range		147.07	
	Interquartile Range		45.27	
	Skewness		.738	.564
	Kurtosis		.380	1.091
PCT_Diff_Histamine_First	Mean		116.2289	8.72675
	95% Confidence Interval for Mean	Lower Bound	97.6283	
		Upper Bound	134.8295	
	5% Trimmed Mean		113.8071	
	Median		103.7091	
	Variance		1218.498	
	Std. Deviation		34.90699	
	Minimum		71.66	
	Maximum		204.39	

	Range		132.73	
	Interquartile Range		55.15	
	Skewness		1.154	.564
	Kurtosis		1.181	1.091
PCT_Diff_Histamine_Second	Mean		135.4181	9.43381
	95% Confidence Interval for Mean	Lower Bound	115.3104	
		Upper Bound	155.5258	
	5% Trimmed Mean		133.2153	
	Median		118.3389	
	Variance		1423.948	
	Std. Deviation		37.73523	
	Minimum		98.14	
	Maximum		212.35	
	Range		114.21	
	Interquartile Range		50.87	
	Skewness		.976	.564
	Kurtosis		-.310	1.091

Tests of Normality

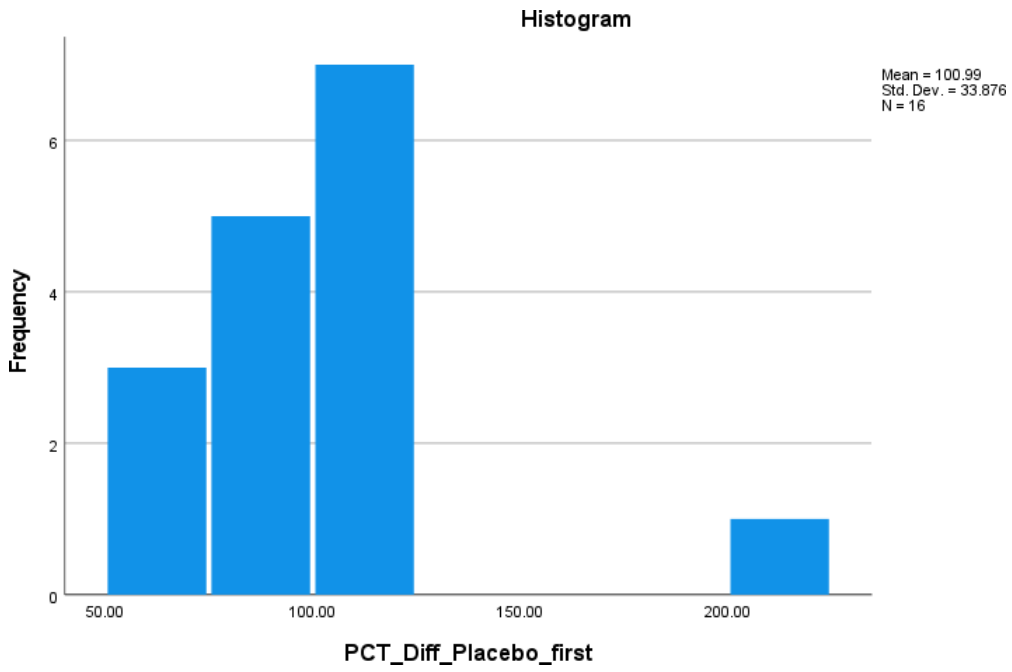
	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
PCT_Diff_Placebo_first	.186	16	.142	.859	16	.018
PCT_Diff_Placebo_Second	.170	16	.200*	.952	16	.521
PCT_Diff_Histamine_First	.201	16	.084	.893	16	.062

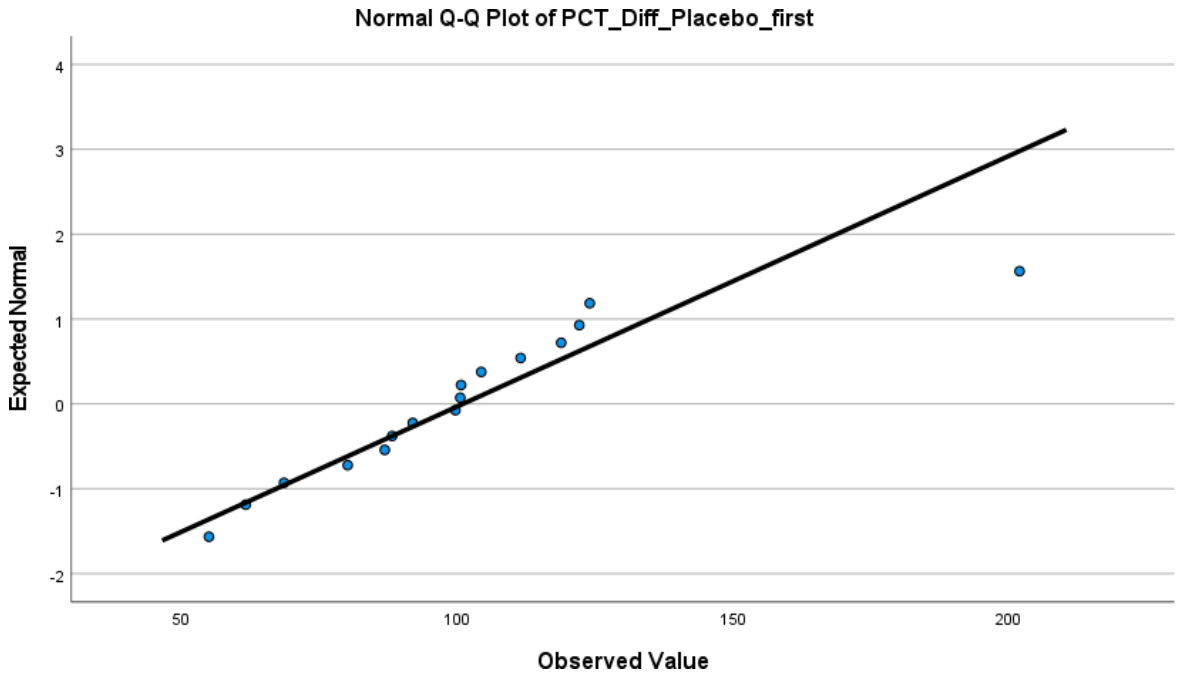
PCT_Diff_Histamine_Second	.228	16	.026	.847	16	.012

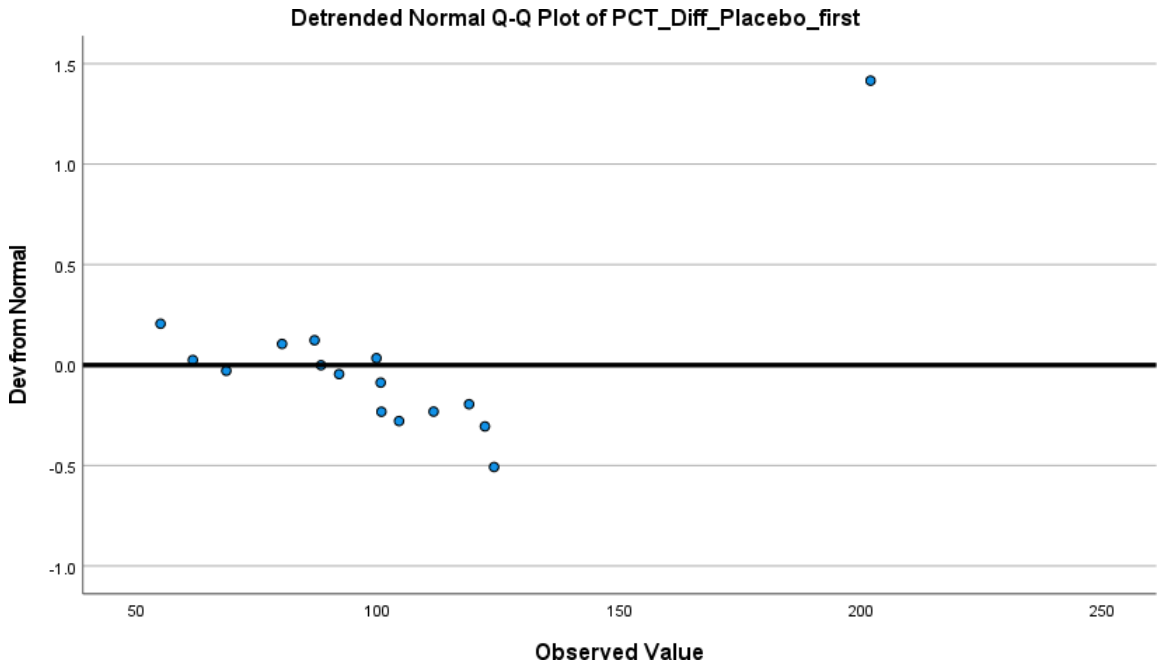
*. This is a lower bound of the true significance.

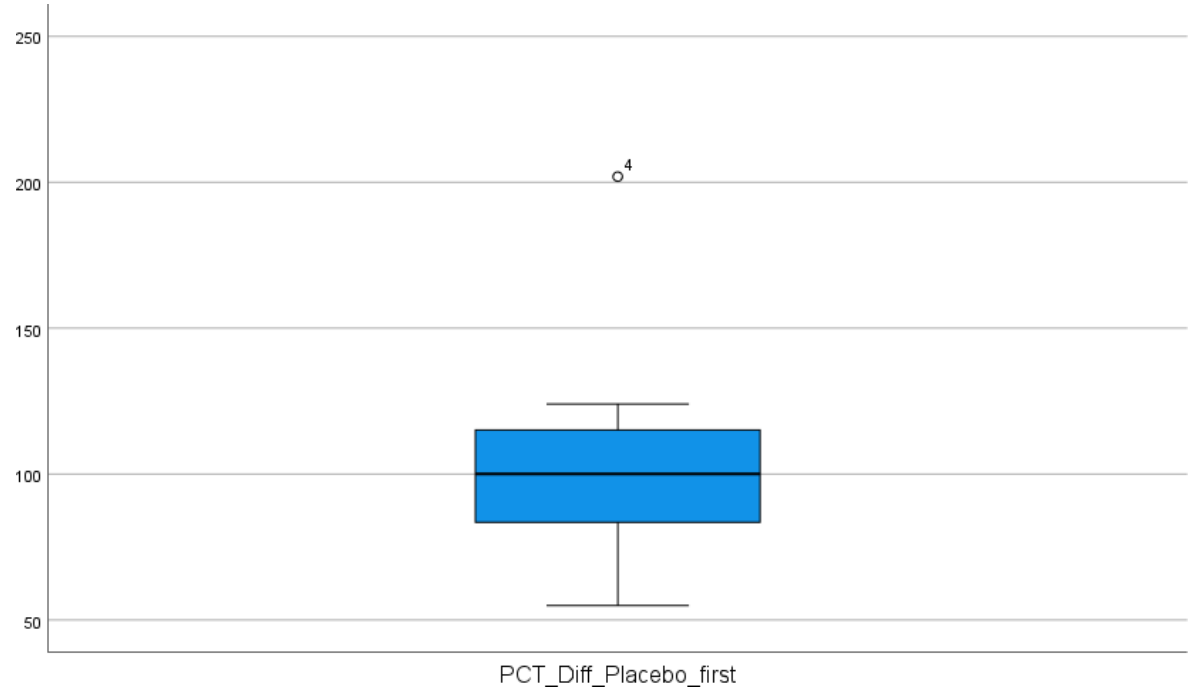
a. Lilliefors Significance Correction

PCT_Diff_Placebo_first

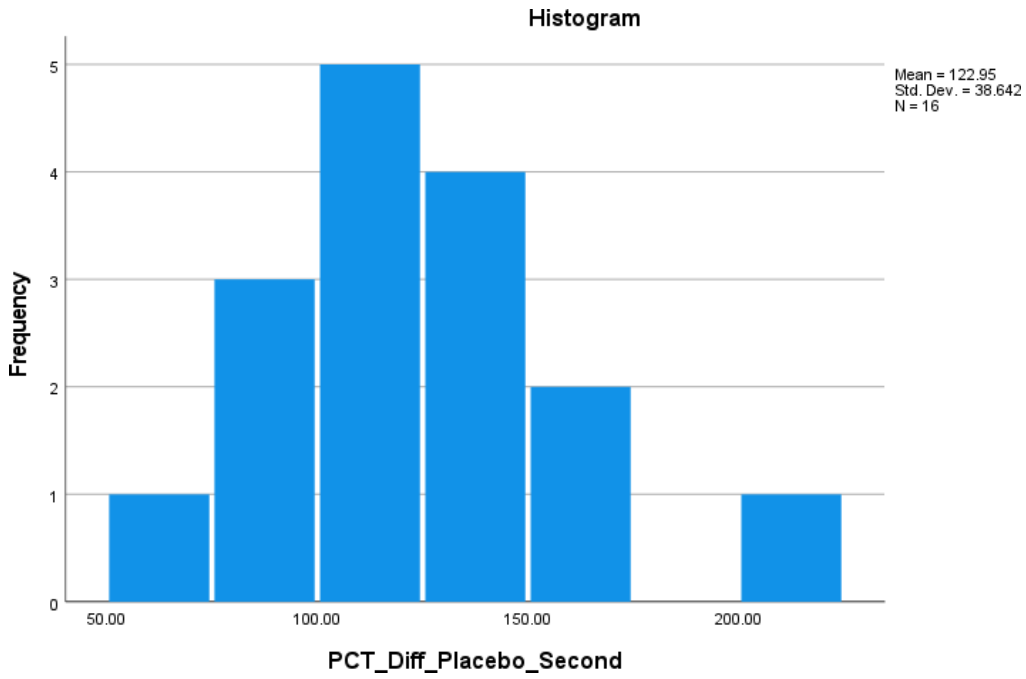


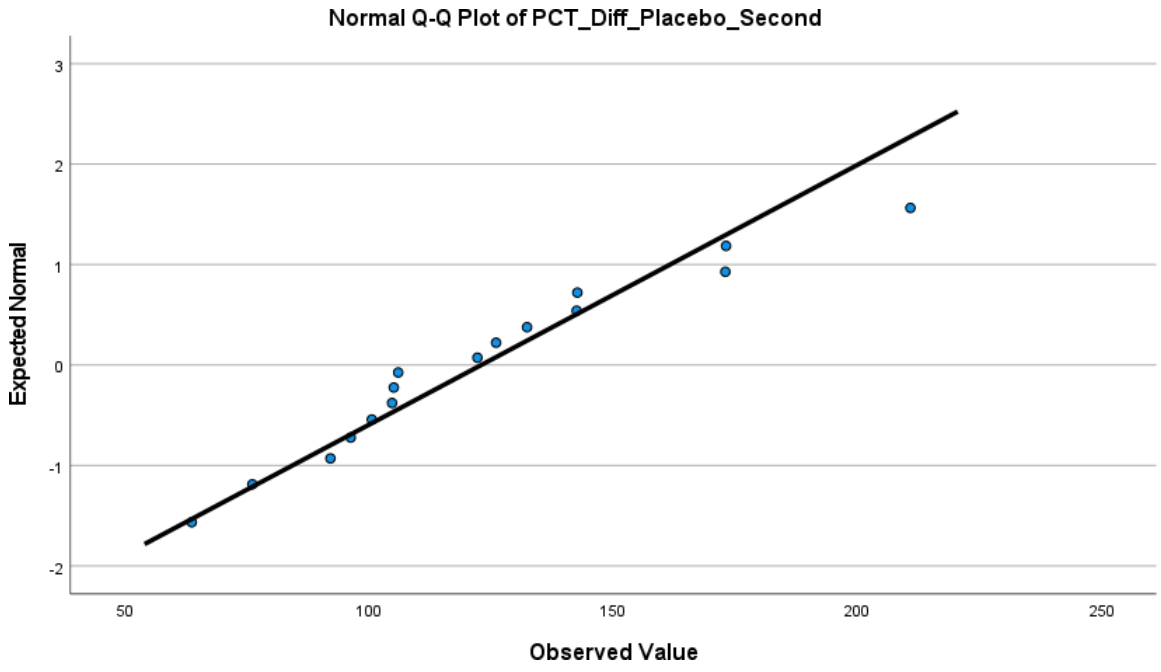

Normal Q-Q Plot of PCT_Diff_Placebo_first
Expected Normal
4
3
2
1
0
-1
-2
50
100
150
200
Observed Value

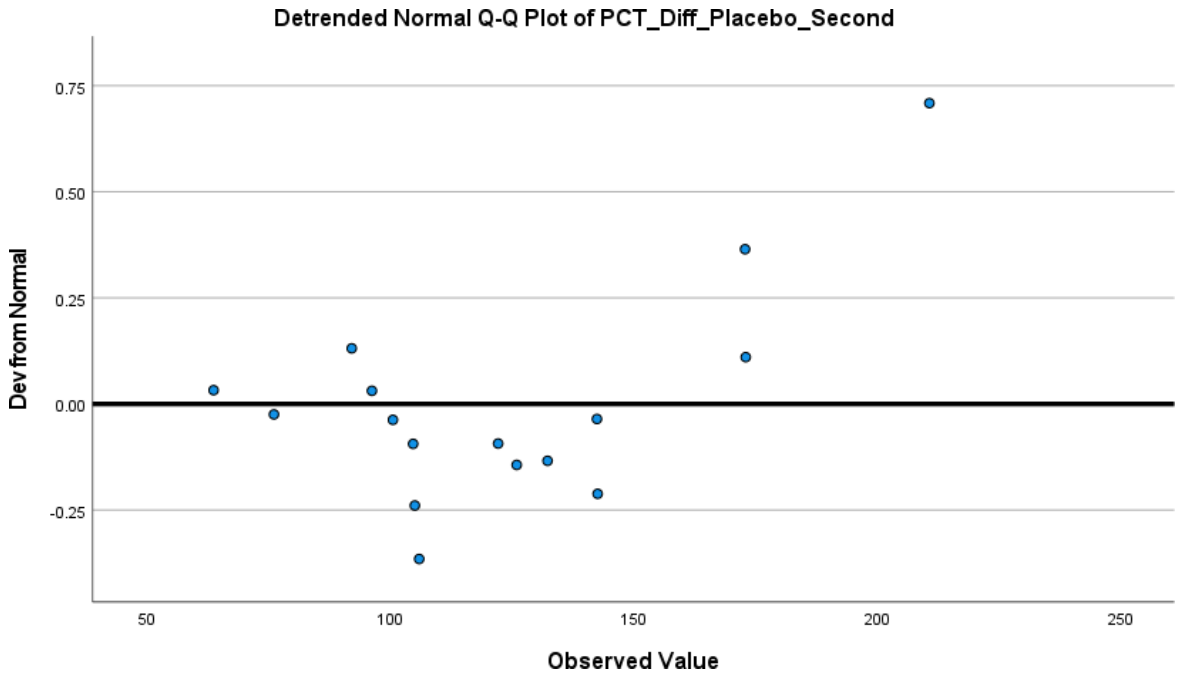

Detrended Normal Q-Q Plot of PCT_Diff_Placebo_first
Dev from Normal
1.5
1.0
0.5
0.0
-0.5
-1.0
50
100
150
200
250
Observed Value

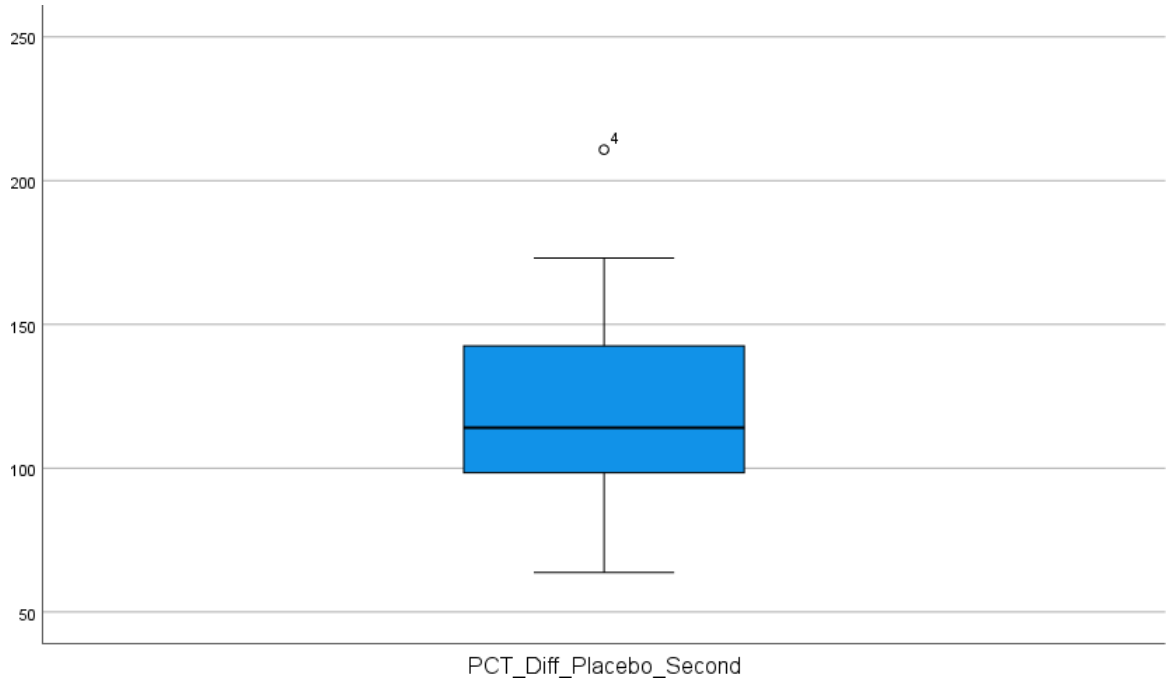


PCT_Diff_Placebo_Second

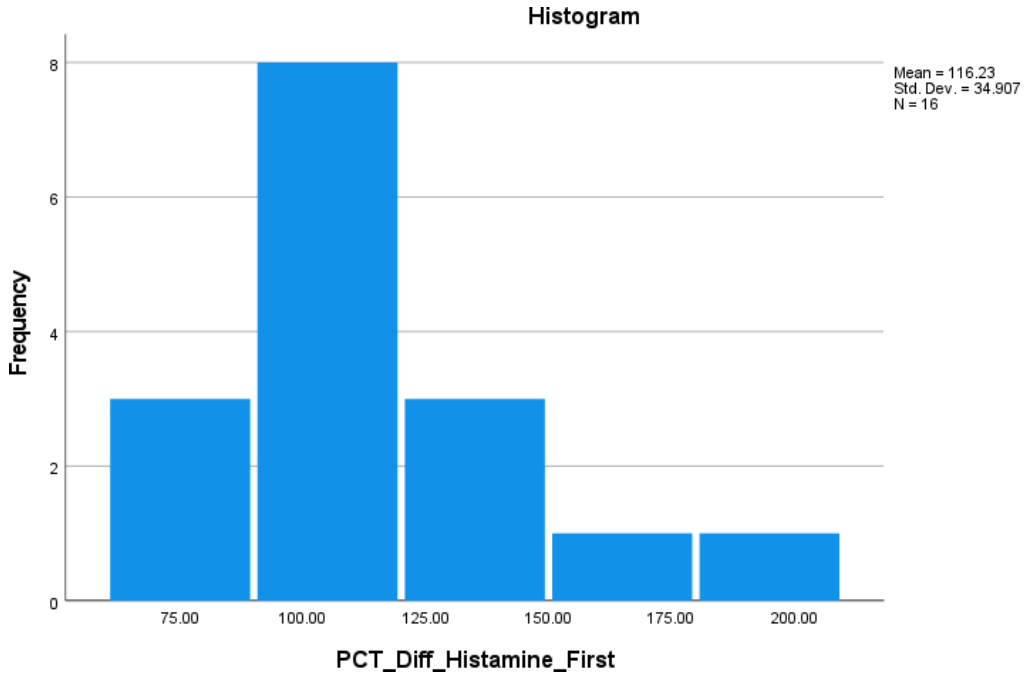

Histogram
Mean = 122.95
Std. Dev. = 38.642
N = 16
Frequency
0
1
2
3
4
5
50.00
100.00
150.00
200.00
PCT_Diff_Placebo_Second

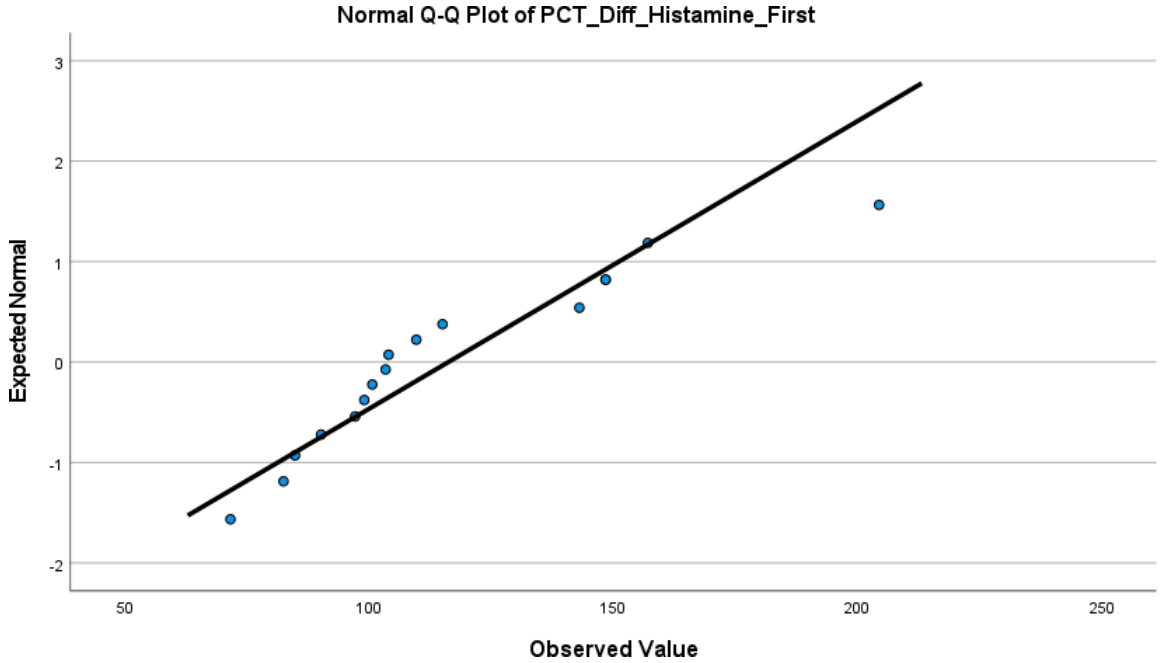

Normal Q-Q Plot of PCT_Diff_Placebo_Second
Expected Normal
3
2
1
0
-1
-2
50
100
150
200
250
Observed Value

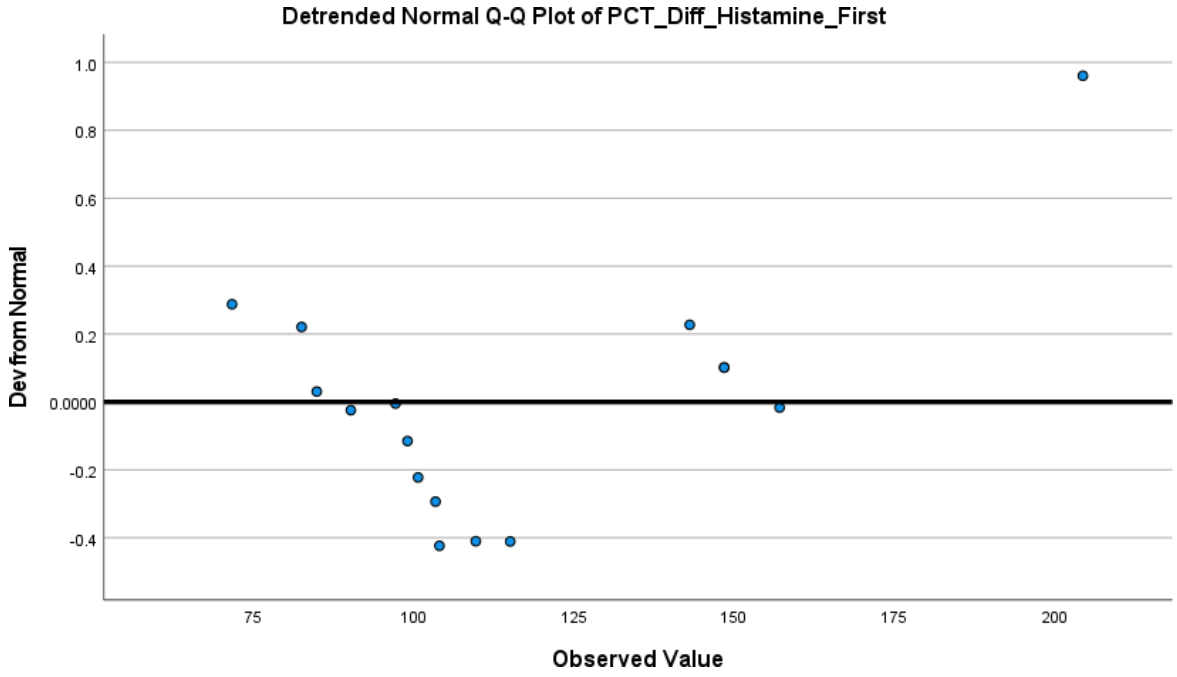

Detrended Normal Q-Q Plot of PCT_Diff_Placebo_Second
Dev from Normal
0.75
0.50
0.25
0.00
-0.25
50
100
150
200
250
Observed Value

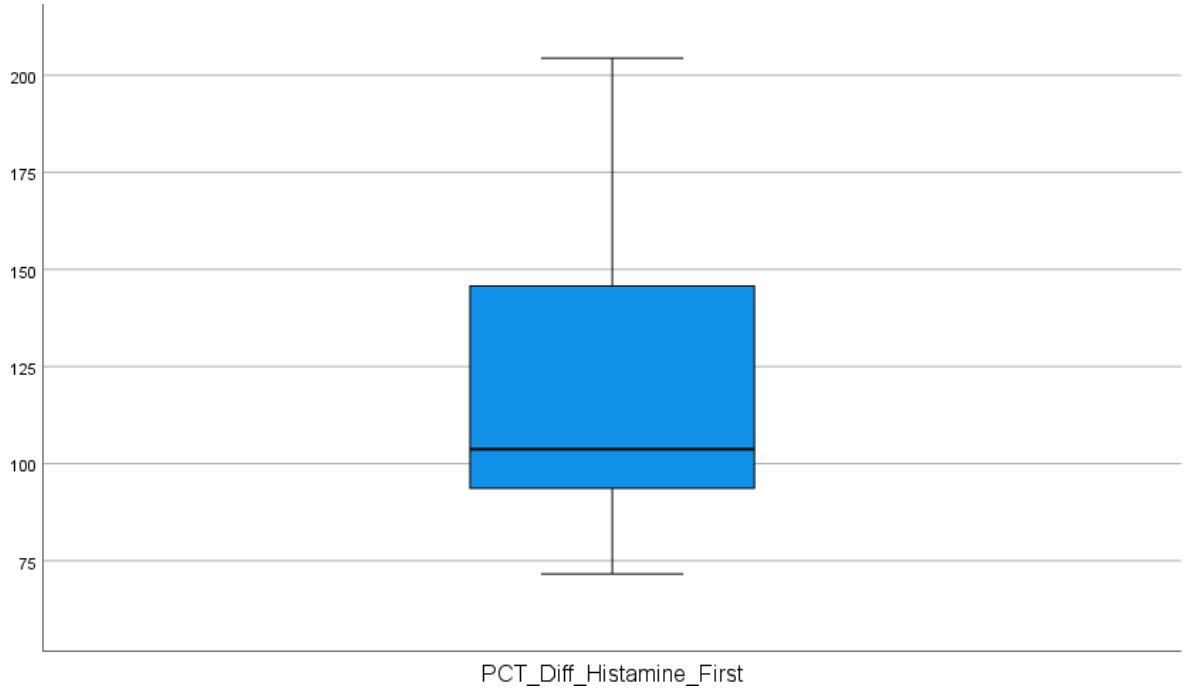

250
200
150
100
50
4
PCT_Diff_Placebo_Second

PCT_Diff_Histamine_First

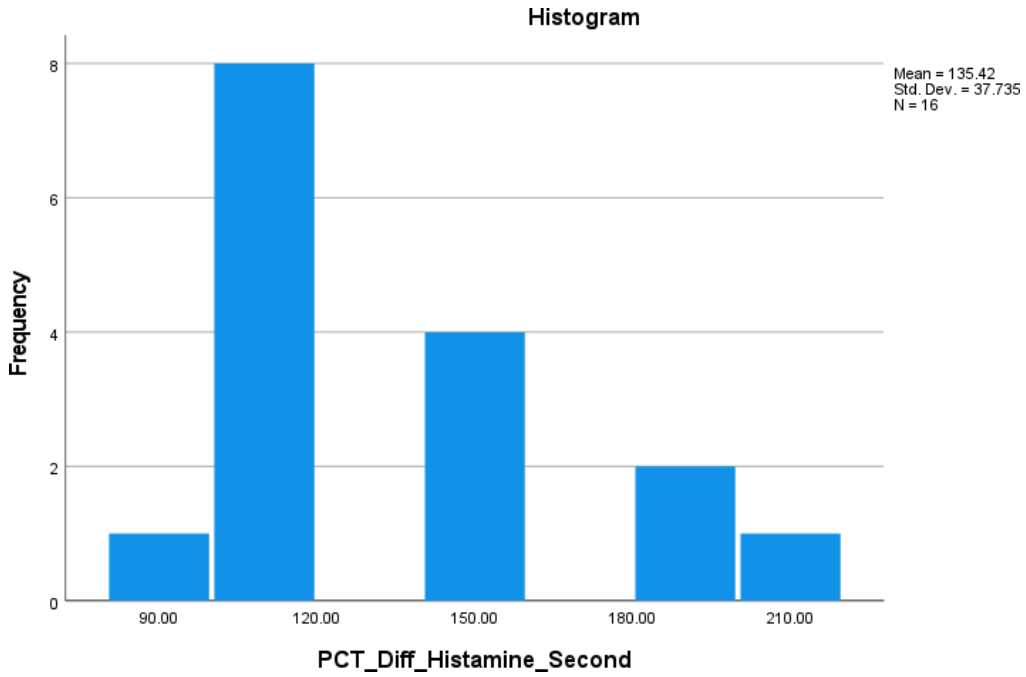


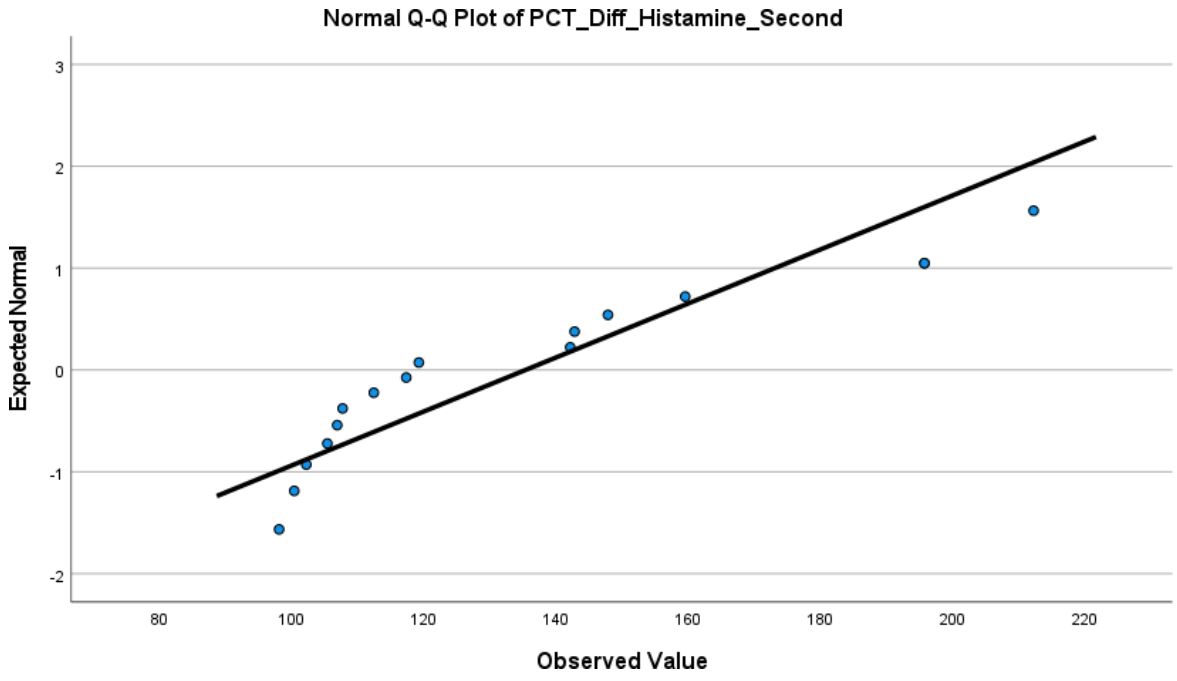


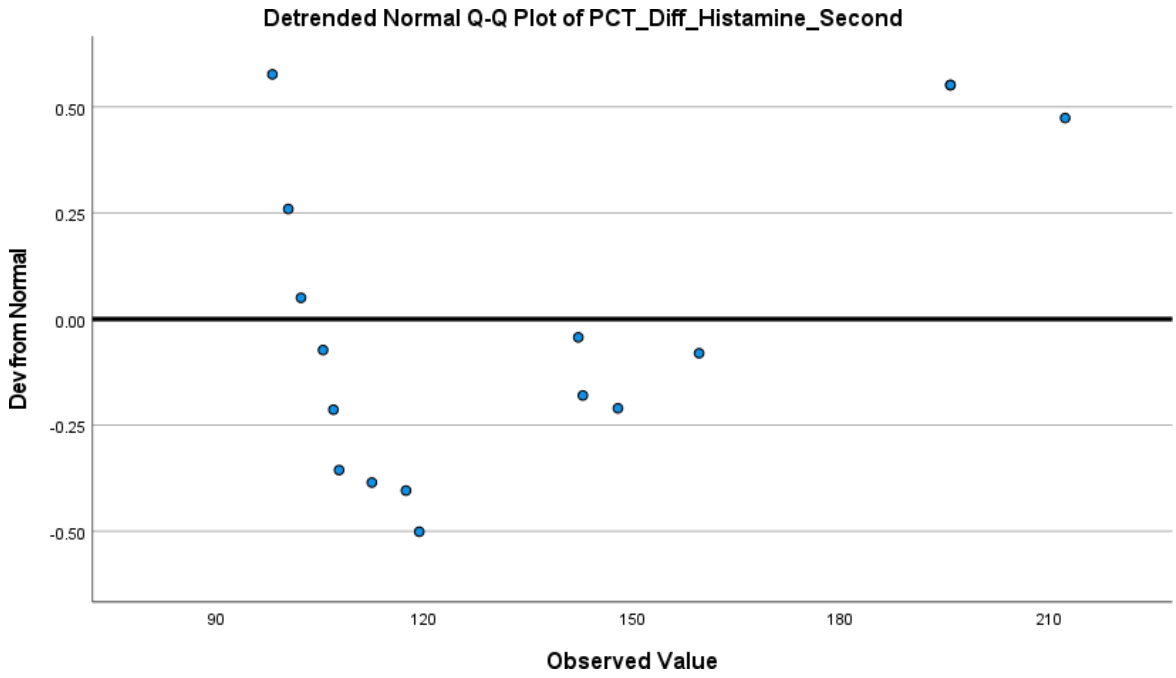

Detrended Normal Q-Q Plot of PCT_Diff_Histamine_First
Dev from Normal
1.0
0.8
0.6
0.4
0.2
0.0000
-0.2
-0.4
75
100
125
150
175
200
Observed Value

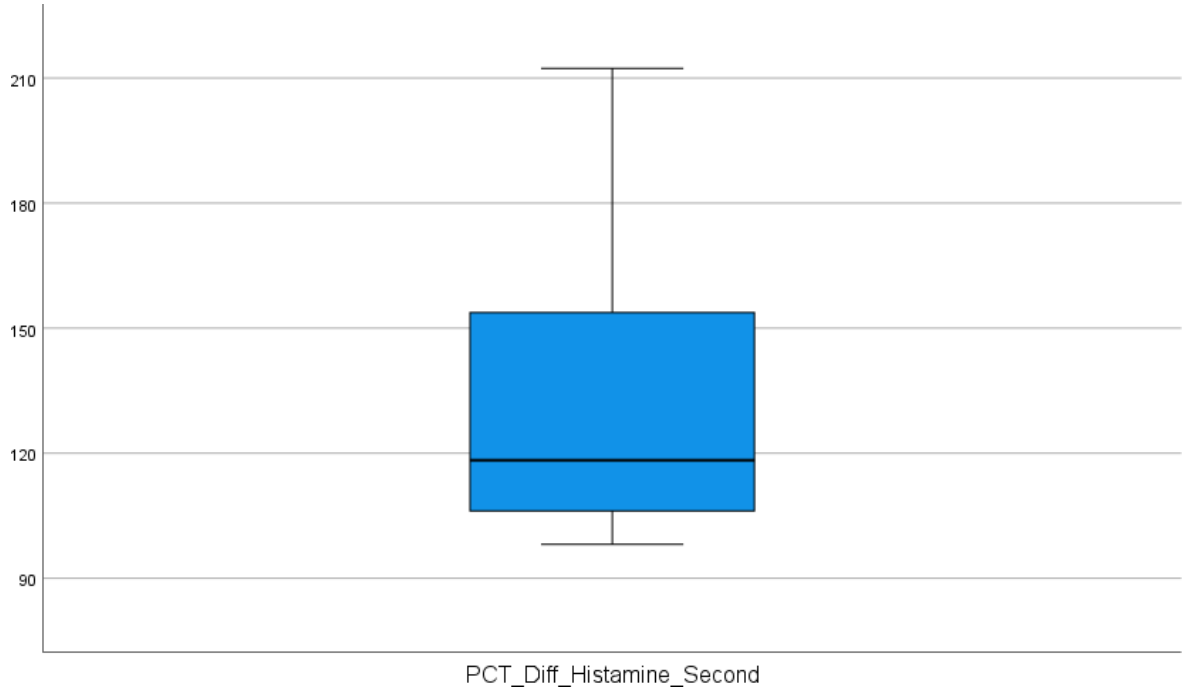

200
175
150
125
100
75
PCT_Diff_Histamine_First

PCT_Diff_Histamine_Second






Detrended Normal Q-Q Plot of PCT_Diff_Histamine_Second
Dev from Normal
0.50
0.25
0.00
-0.25
-0.50
90
120
150
180
210
Observed Value


210
180
150
120
90
PCT_Diff_Histamine_Second

Appendix J

Nonparametric Tests_Study2_contra

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.[a,b]
1	The median of PCT_Diff_Placebo_first equals 100.00.	One-Sample Wilcoxon Signed Rank Test	.140
2	The median of PCT_Diff_Placebo_Second equals 100.00.	One-Sample Wilcoxon Signed Rank Test	.778
3	The median of PCT_Diff_Histamine_First equals 100.00.	One-Sample Wilcoxon Signed Rank Test	.594
4	The median of PCT_Diff_Histamine_Second equals 100.00.	One-Sample Wilcoxon Signed Rank Test	.041

Hypothesis Test Summary

	Decision
1	Retain the null hypothesis.
2	Retain the null hypothesis.
3	Retain the null hypothesis.
4	Reject the null hypothesis.

a. The significance level is .050.

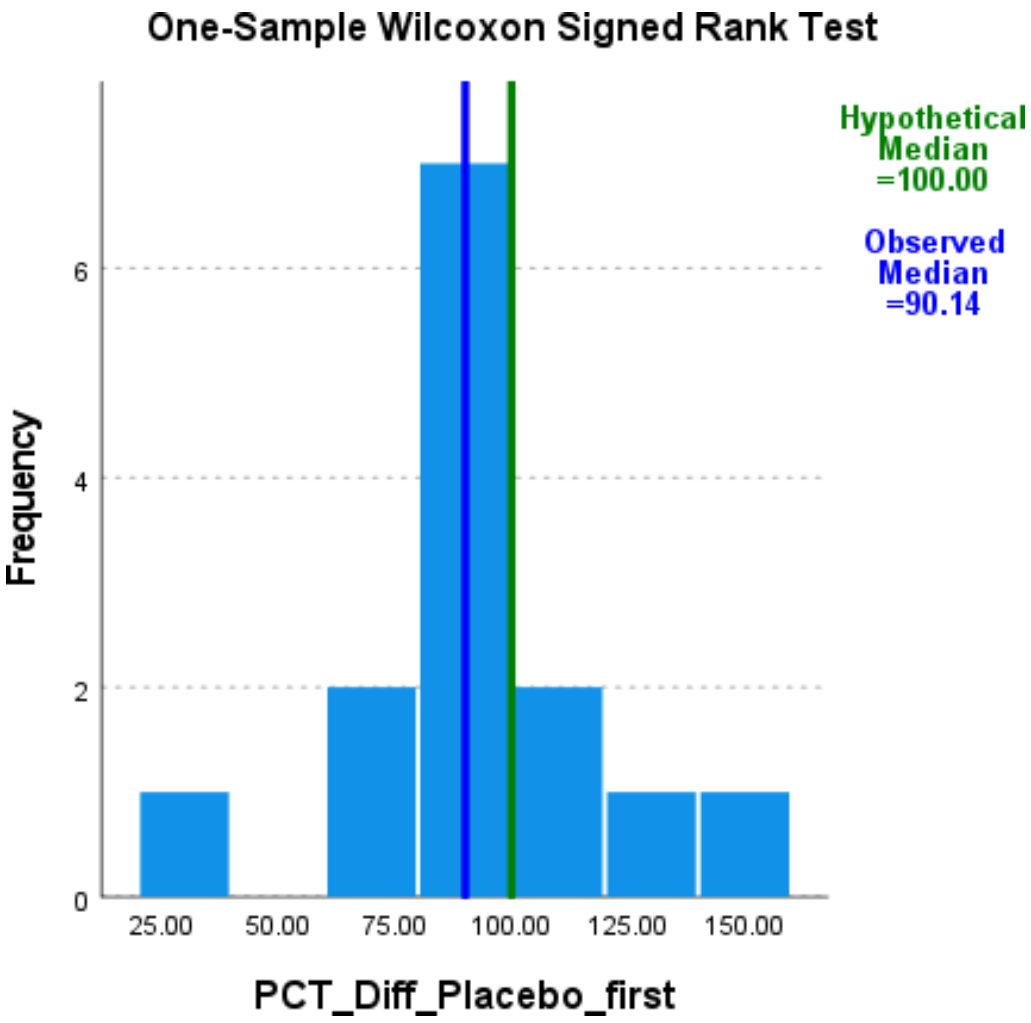
b. Asymptotic significance is displayed.

One-Sample Wilcoxon Signed Rank Test

PCT_Diff_Placebo_first

One-Sample Wilcoxon Signed Rank Test Summary

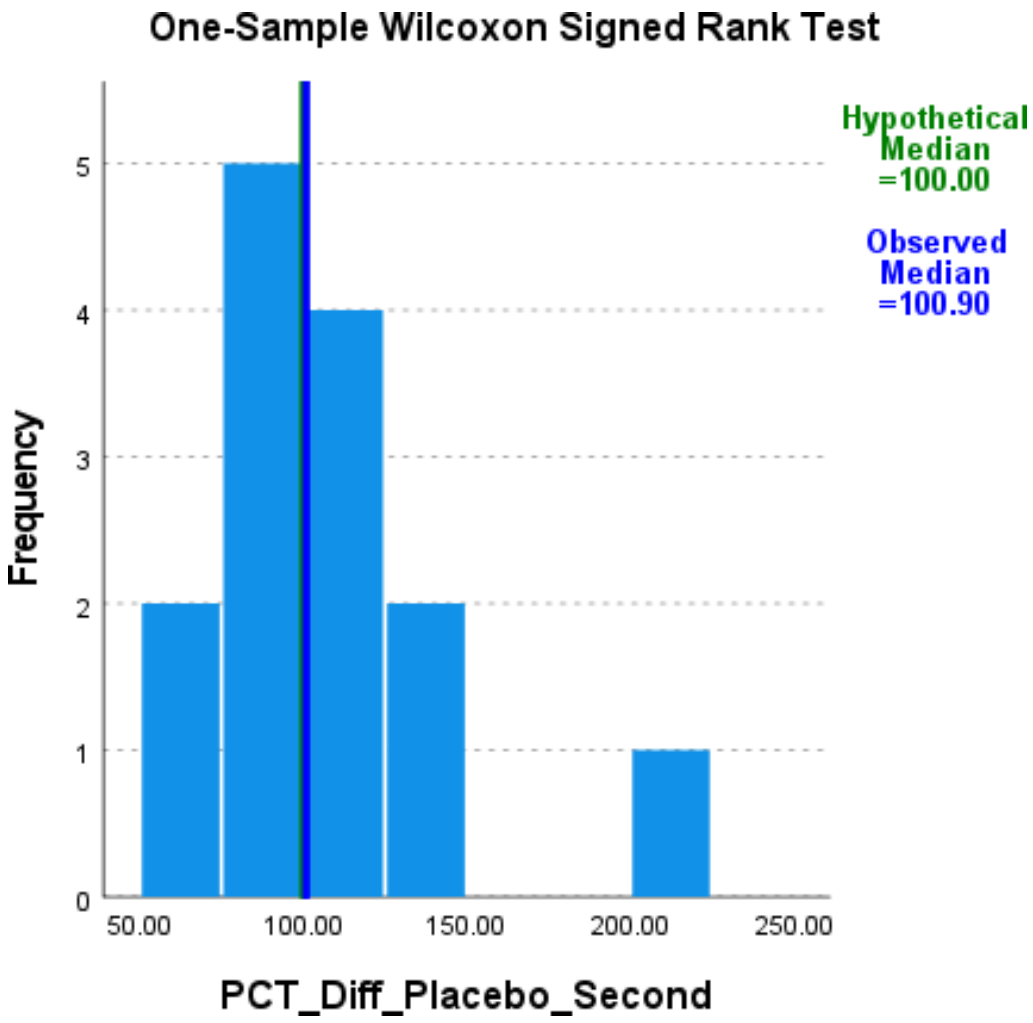
Total N	14
Test Statistic	29.000
Standard Error	15.930
Standardized Test Statistic	-1.475
Asymptotic Sig.(2-sided test)	.140


One-Sample Wilcoxon Signed Rank Test
Hypothetical Median =100.00
Observed Median =90.14
Frequency
0
2
4
6
25.00
50.00
75.00
100.00
125.00
150.00
PCT_Diff_Placebo_first

PCT_Diff_Placebo_Second

One-Sample Wilcoxon Signed Rank Test Summary

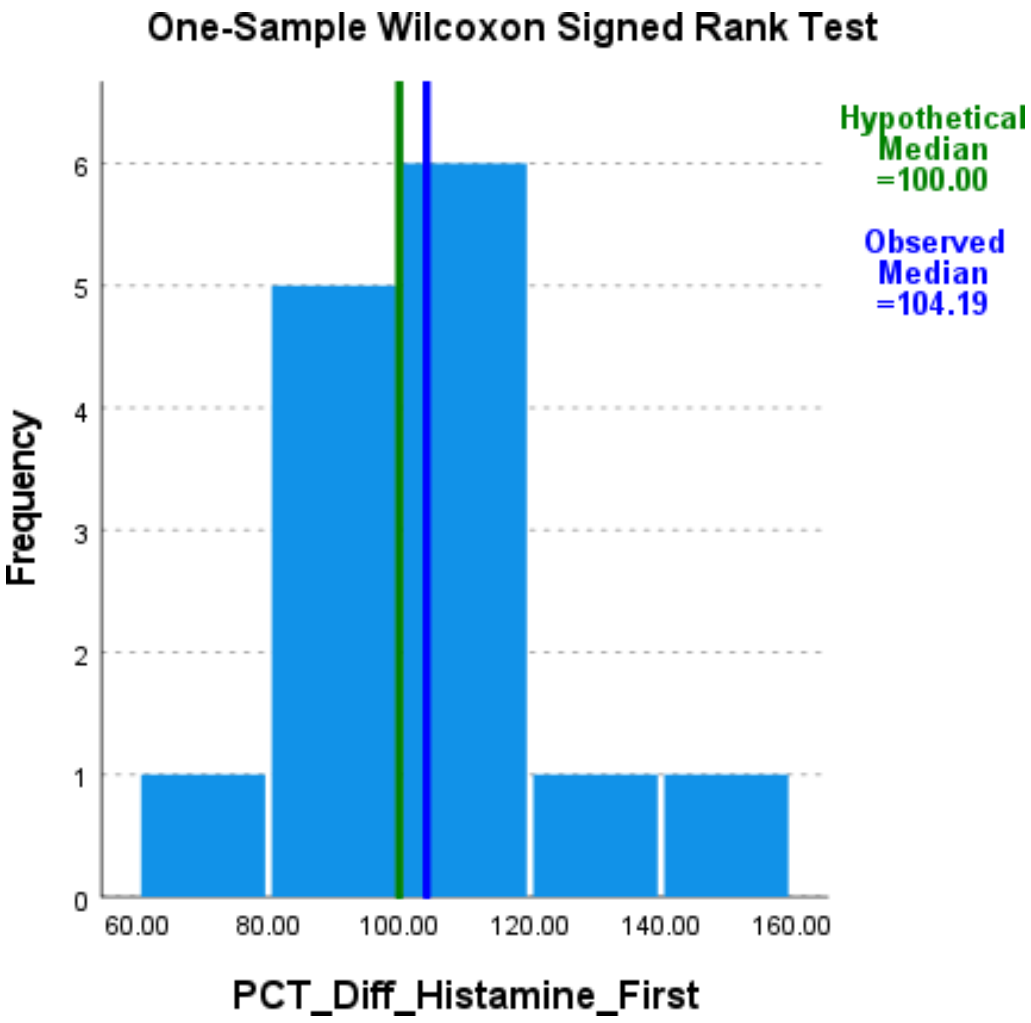
Total N	14
Test Statistic	57.000
Standard Error	15.930
Standardized Test Statistic	.282
Asymptotic Sig.(2-sided test)	.778


One-Sample Wilcoxon Signed Rank Test
Hypothetical Median =100.00
Observed Median =100.90
Frequency
0
1
2
3
4
5
50.00
100.00
150.00
200.00
250.00
PCT_Diff_Placebo_Second

PCT_Diff_Histamine_First

One-Sample Wilcoxon Signed Rank Test Summary

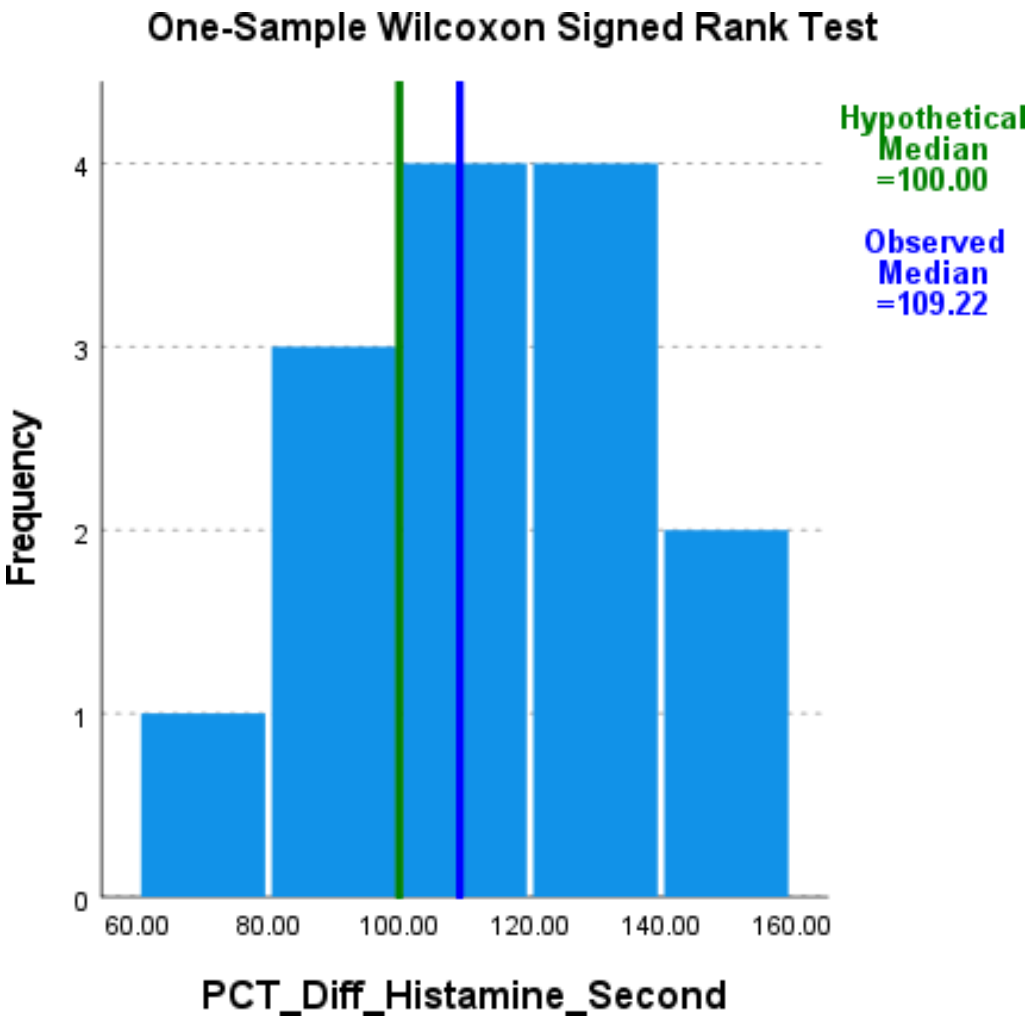
Total N	14
Test Statistic	61.000
Standard Error	15.930
Standardized Test Statistic	.534
Asymptotic Sig.(2-sided test)	.594

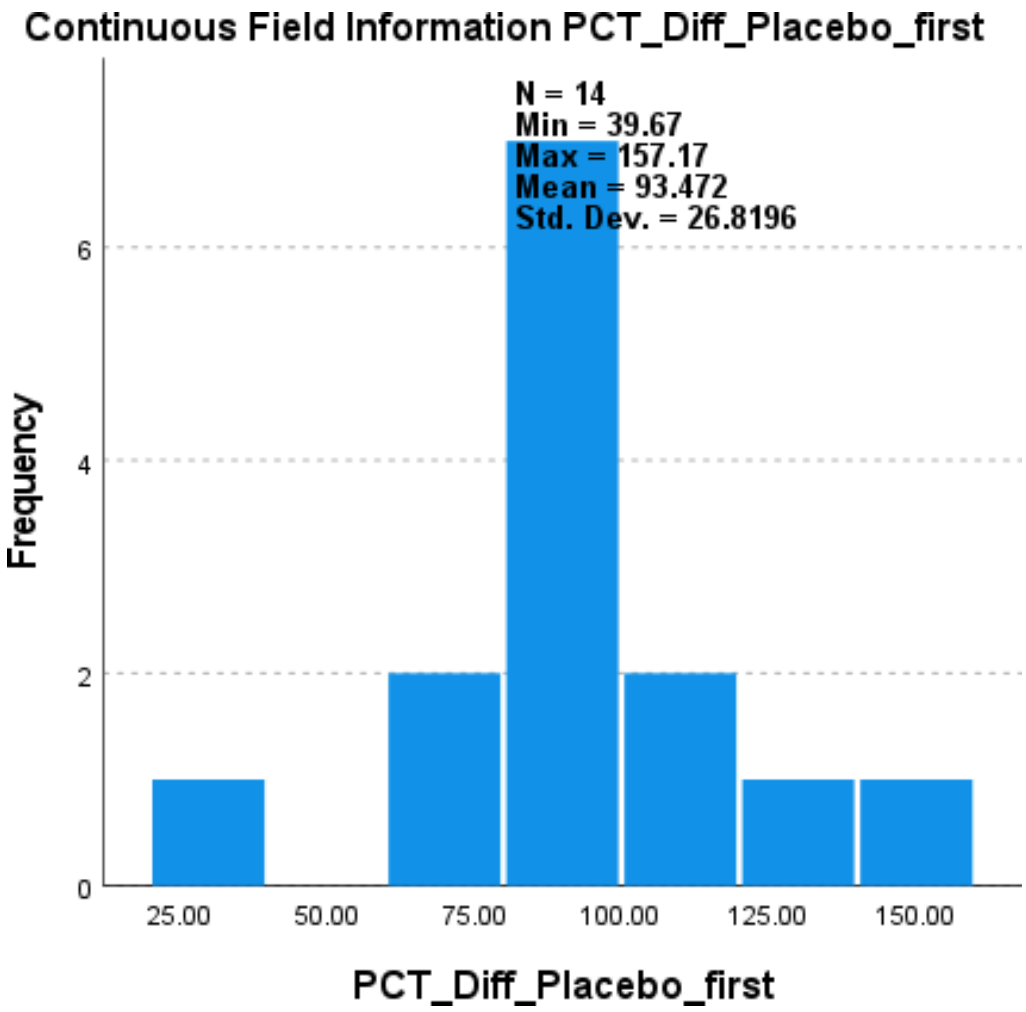

One-Sample Wilcoxon Signed Rank Test
Hypothetical Median =100.00
Observed Median =104.19
Frequency
0
1
2
3
4
5
6
60.00
80.00
100.00
120.00
140.00
160.00
PCT_Diff_Histamine_First

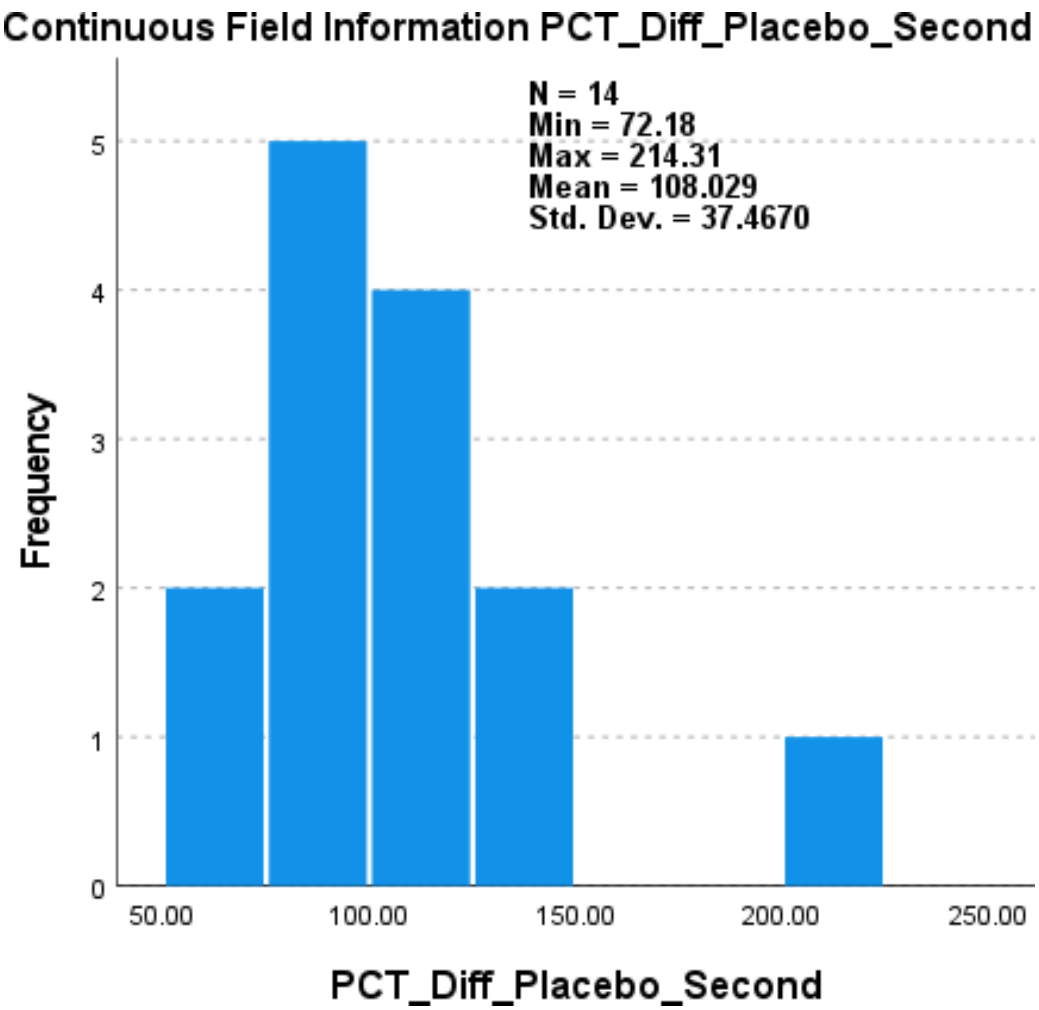
PCT_Diff_Histamine_Second

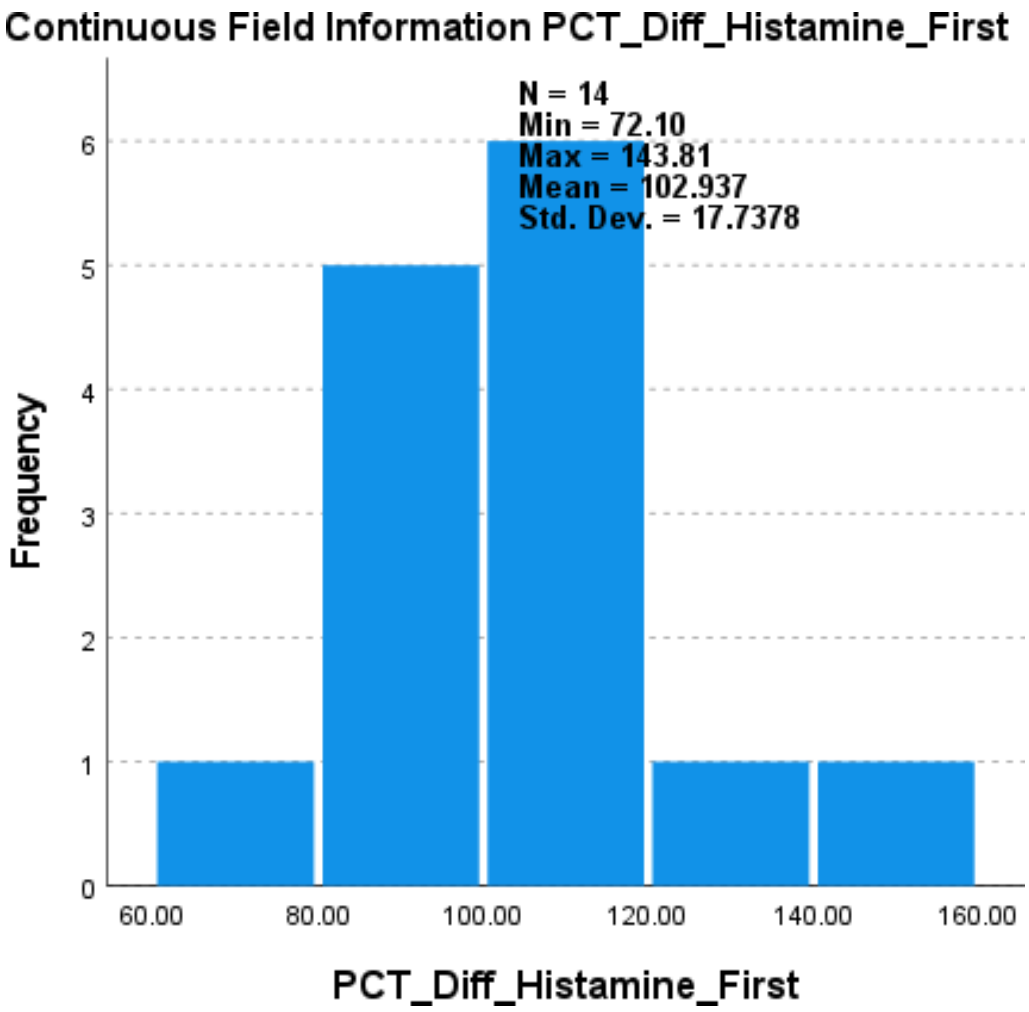
One-Sample Wilcoxon Signed Rank Test Summary

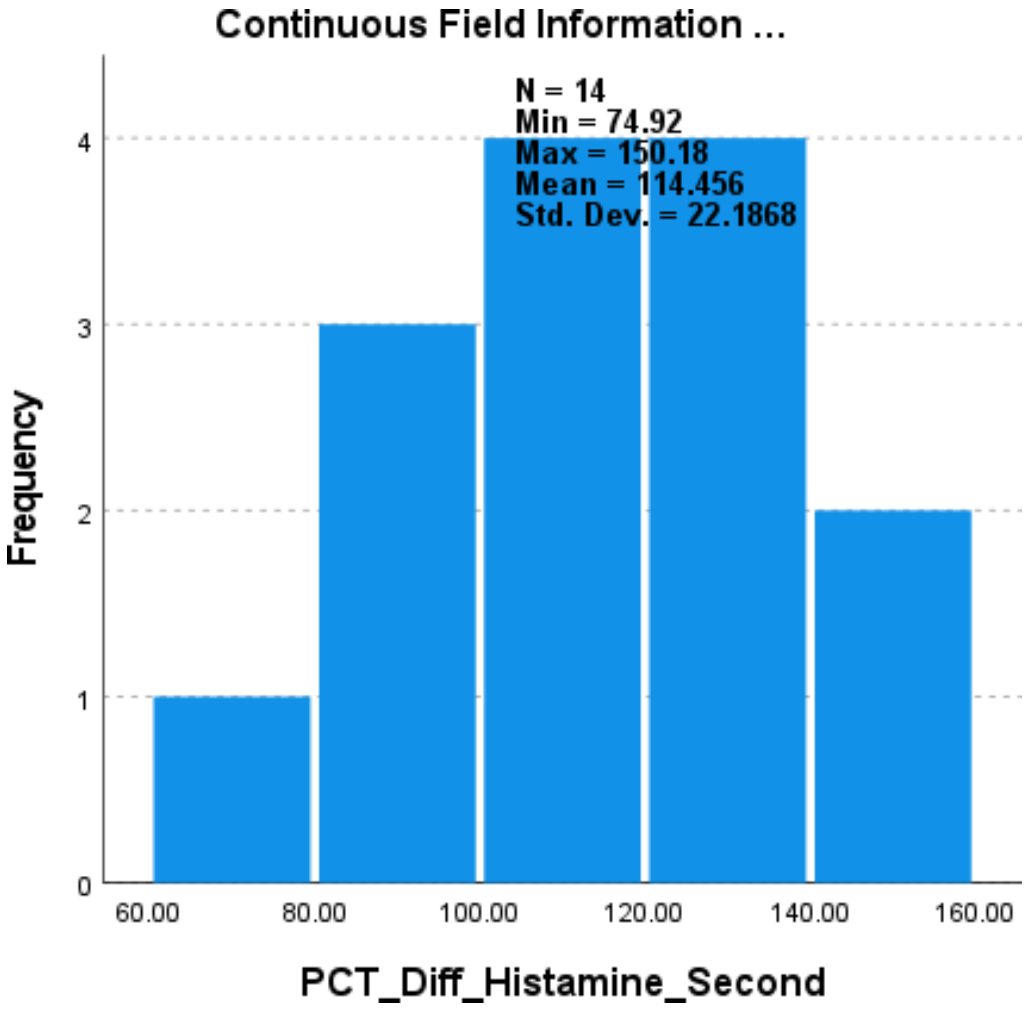
Total N	14
Test Statistic	85.000
Standard Error	15.930
Standardized Test Statistic	2.040
Asymptotic Sig.(2-sided test)	.041


One-Sample Wilcoxon Signed Rank Test
Hypothetical Median =100.00
Observed Median =109.22
Frequency
0
1
2
3
4
60.00
80.00
100.00
120.00
140.00
160.00
PCT_Diff_Histamine_Second


Continuous Field Information PCT_Diff_Placebo_first
N = 14
Min = 39.67
Max = 157.17
Mean = 93.472
Std. Dev. = 26.8196
Frequency
0
2
4
6
25.00
50.00
75.00
100.00
125.00
150.00
PCT_Diff_Placebo_first


Continuous Field Information PCT_Diff_Placebo_Second
N = 14
Min = 72.18
Max = 214.31
Mean = 108.029
Std. Dev. = 37.4670
Frequency
0
1
2
3
4
5
50.00
100.00
150.00
200.00
250.00
PCT_Diff_Placebo_Second


Continuous Field Information PCT_Diff_Histamine_First
N = 14
Min = 72.10
Max = 143.81
Mean = 102.937
Std. Dev. = 17.7378
Frequency
0
1
2
3
4
5
6
60.00
80.00
100.00
120.00
140.00
160.00
PCT_Diff_Histamine_First


Continuous Field Information ...
N = 14
Min = 74.92
Max = 150.18
Mean = 114.456
Std. Dev. = 22.1868
Frequency
0
1
2
3
4
60.00
80.00
100.00
120.00
140.00
160.00
PCT_Diff_Histamine_Second

NPar Tests

Wilcoxon Signed Ranks Test

Ranks

		N	Mean Rank	Sum of Ranks
P_halves_mean - H_halves_mean	Negative Ranks	9[a]	7.89	71.00
	Positive Ranks	5[b]	6.80	34.00
	Ties	0[c]		
	Total	14		
second_half_mean - first_half_mean	Negative Ranks	3[d]	4.00	12.00
	Positive Ranks	11[e]	8.45	93.00
	Ties	0[f]		
	Total	14		

a. P_halves_mean < H_halves_mean

b. P_halves_mean > H_halves_mean

c. P_halves_mean = H_halves_mean

d. second_half_mean < first_half_mean

e. second_half_mean > first_half_mean

f. second_half_mean = first_half_mean

Test Statistics[a]

	P_halves_mean - H_halves_mean	second_half_mean - first_half_mean
Z	-1.161[b]	-2.542[c]
Asymp. Sig. (2-tailed)	.245	.011

a. Wilcoxon Signed Ranks Test

b. Based on positive ranks.

c. Based on negative ranks.

Nonparametric Tests

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.[a,b]
1	The median of interaction equals .00.	One-Sample Wilcoxon Signed Rank Test	.510

Hypothesis Test Summary

	Decision
1	Retain the null hypothesis.

a. The significance level is .050.

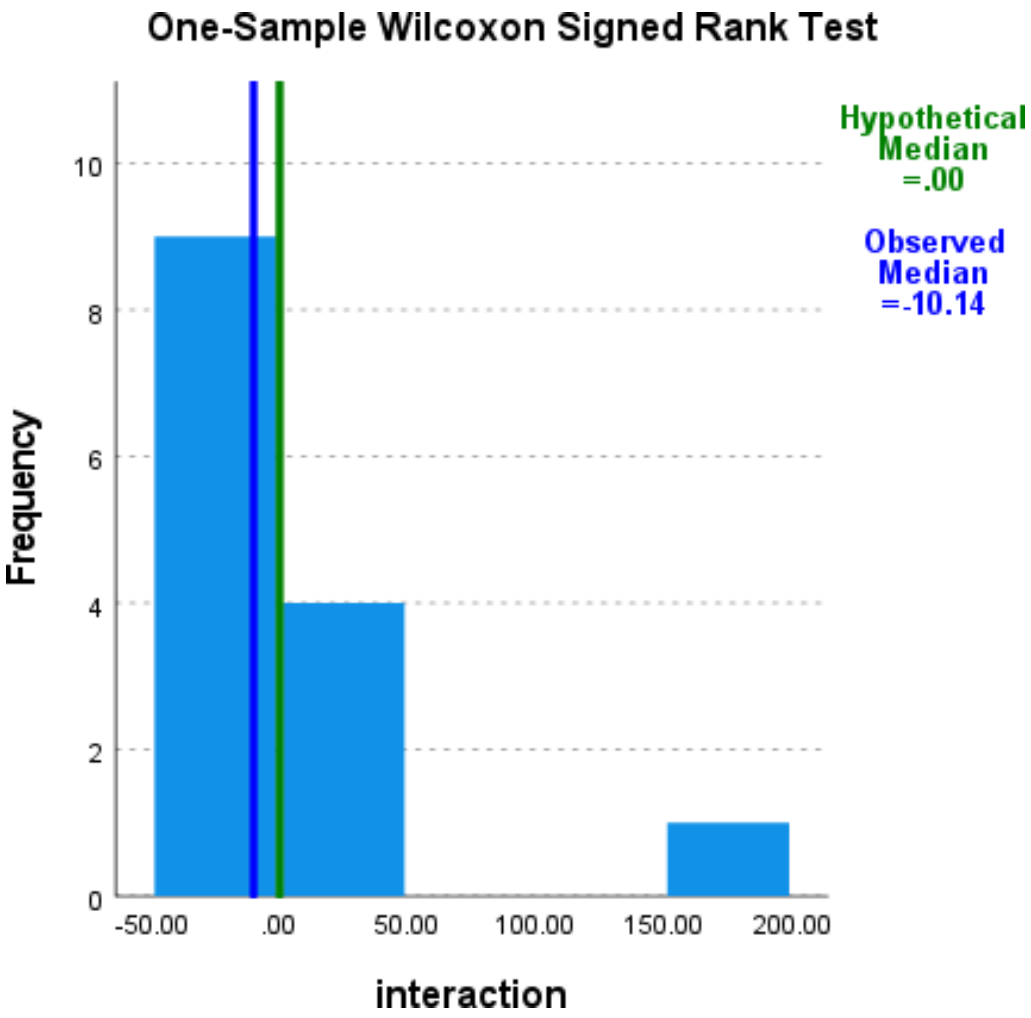
b. Asymptotic significance is displayed.

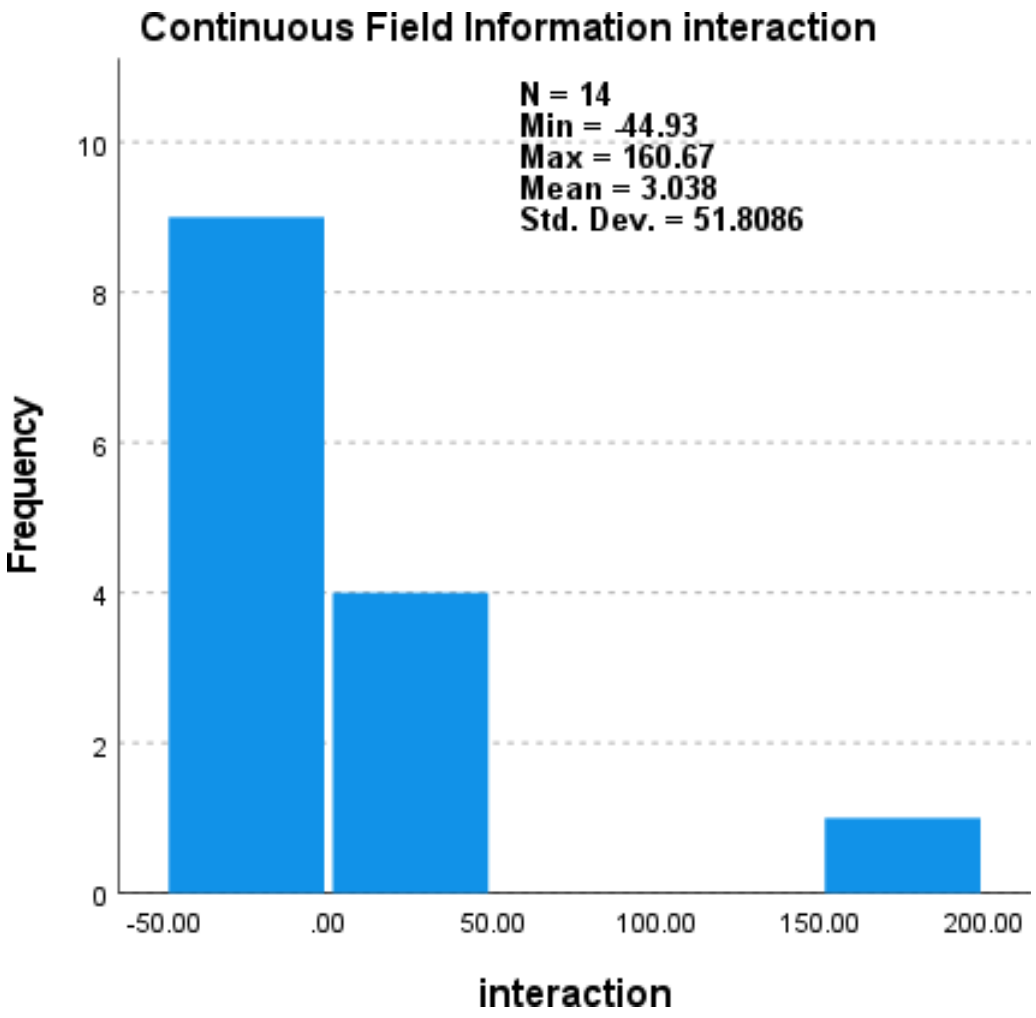
One-Sample Wilcoxon Signed Rank Test

interaction

One-Sample Wilcoxon Signed Rank Test Summary

Total N	14
Test Statistic	42.000
Standard Error	15.930
Standardized Test Statistic	-.659
Asymptotic Sig.(2-sided test)	.510


One-Sample Wilcoxon Signed Rank Test
Hypothetical Median =.00
Observed Median =-10.14
Frequency
0
2
4
6
8
10
-50.00
.00
50.00
100.00
150.00
200.00
interaction



DESCRIPTIVES VARIABLES=PCT_Diff_Placebo_first PCT_Diff_Placebo_Second PCT_Diff_Histamine_First

PCT_Diff_Histamine_Second

/STATISTICS=MEAN STDDEV MIN MAX.

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
PCT_Diff_Placebo_first	14	39.67	157.17	93.4721	26.81963
PCT_Diff_Placebo_Second	14	72.18	214.31	108.0288	37.46704
PCT_Diff_Histamine_First	14	72.10	143.81	102.9369	17.73779
PCT_Diff_Histamine_Second	14	74.92	150.18	114.4557	22.18676
Valid N (listwise)	14				

Statistics

		PCT_Diff_Placebo_first	PCT_Diff_Placebo_Second	PCT_Diff_Histamine_First	PCT_Diff_Histamine_Second
N	Valid	14	14	14	14
	Missing	0	0	0	0
Mean		93.4721	108.0288	102.9369	114.4557
Median		90.1410	100.9033	104.1883	109.2206
Std. Deviation		26.81963	37.46704	17.73779	22.18676

Explore

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
PCT_Diff_Placebo_first	14	100.0%	0	0.0%	14	100.0%
PCT_Diff_Placebo_Second	14	100.0%	0	0.0%	14	100.0%
PCT_Diff_Histamine_First	14	100.0%	0	0.0%	14	100.0%
PCT_Diff_Histamine_Second	14	100.0%	0	0.0%	14	100.0%

Descriptives

	Statistic	Std. Error

PCT_Diff_Placebo_first	Mean		93.4721	7.16785
	95% Confidence Interval for Mean	Lower Bound	77.9869	
		Upper Bound	108.9573	
	5% Trimmed Mean		92.9224	
	Median		90.1410	
	Variance		719.292	
	Std. Deviation		26.81963	
	Minimum		39.67	
	Maximum		157.17	
	Range		117.50	
	Interquartile Range		19.79	
	Skewness		.598	.597
	Kurtosis		2.462	1.154
PCT_Diff_Placebo_Second	Mean		108.0288	10.01349
	95% Confidence Interval for Mean	Lower Bound	86.3960	
		Upper Bound	129.6616	
	5% Trimmed Mean		104.1159	
	Median		100.9033	
	Variance		1403.779	
	Std. Deviation		37.46704	
	Minimum		72.18	
	Maximum		214.31	
	Range		142.13	
	Interquartile Range		36.32	
	Skewness		1.919	.597

	Kurtosis		4.507	1.154
PCT_Diff_Histamine_First	Mean		102.9369	4.74062
	95% Confidence Interval for Mean	Lower Bound	92.6954	
		Upper Bound	113.1783	
	5% Trimmed Mean		102.3791	
	Median		104.1883	
	Variance		314.629	
	Std. Deviation		17.73779	
	Minimum		72.10	
	Maximum		143.81	
	Range		71.72	
	Interquartile Range		24.29	
	Skewness		.580	.597
	Kurtosis		1.182	1.154
PCT_Diff_Histamine_Second	Mean		114.4557	5.92966
	95% Confidence Interval for Mean	Lower Bound	101.6454	
		Upper Bound	127.2659	
	5% Trimmed Mean		114.6671	
	Median		109.2206	
	Variance		492.252	
	Std. Deviation		22.18676	
	Minimum		74.92	
	Maximum		150.18	
	Range		75.26	
	Interquartile Range		38.19	

	Skewness	.045	.597
	Kurtosis	-.704	1.154

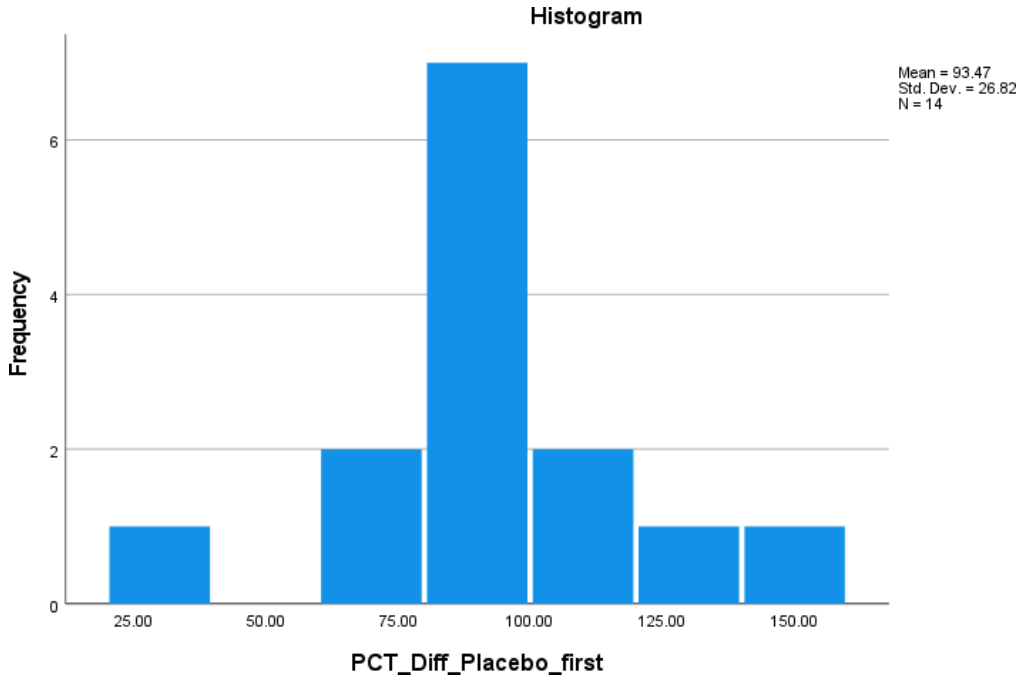
Tests of Normality

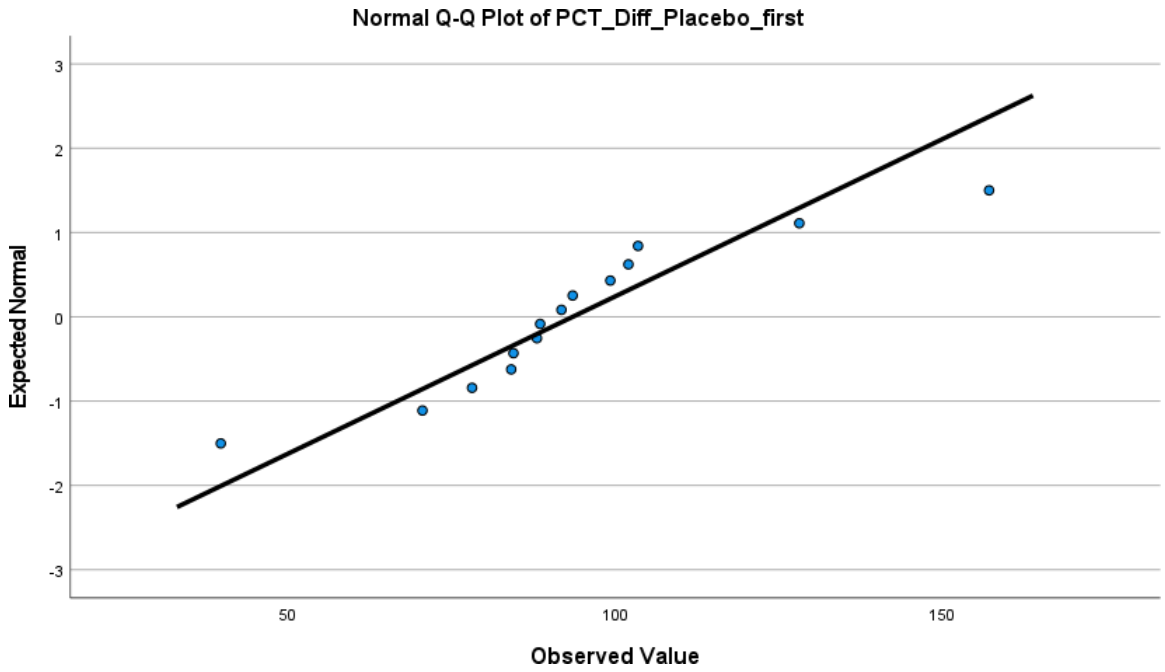
	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
PCT_Diff_Placebo_first	.212	14	.089	.917	14	.200
PCT_Diff_Placebo_Second	.249	14	.019	.809	14	.006
PCT_Diff_Histamine_First	.146	14	.200*	.970	14	.874
PCT_Diff_Histamine_Second	.161	14	.200*	.968	14	.845

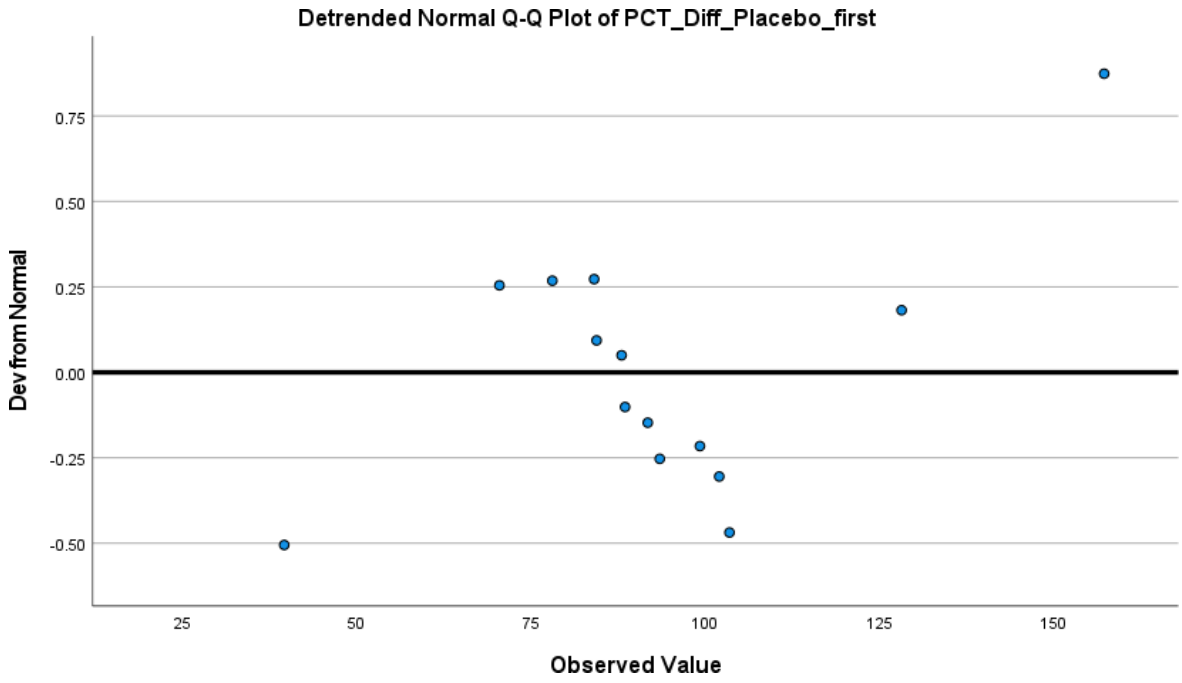
*. This is a lower bound of the true significance.

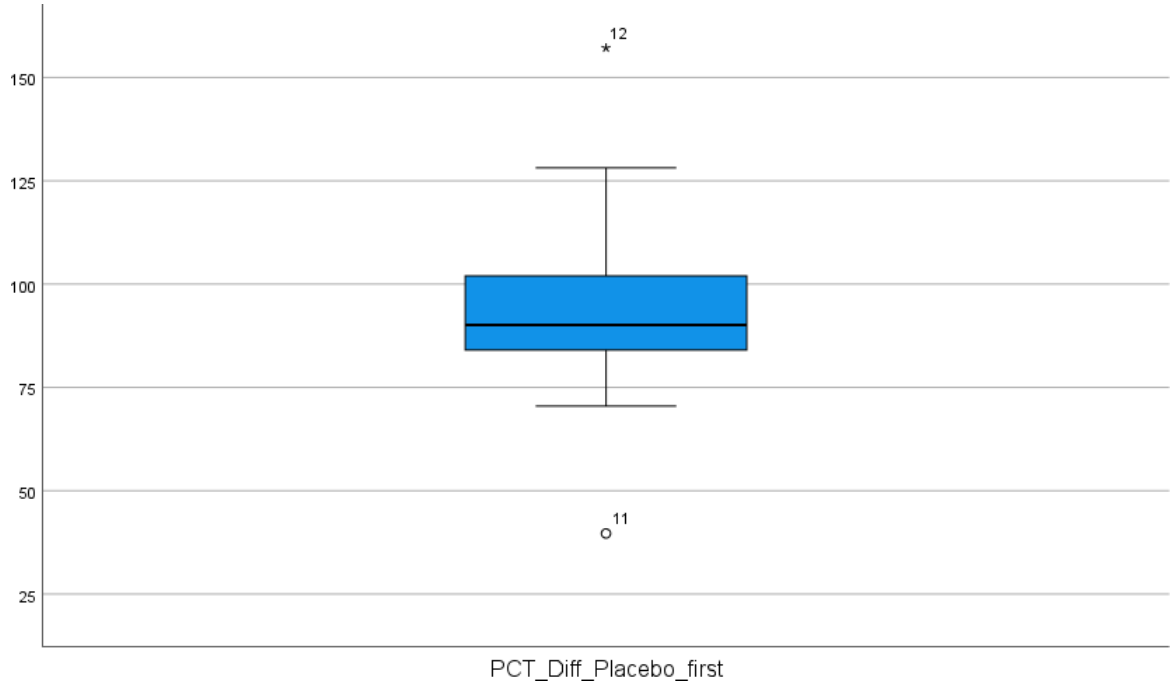
a. Lilliefors Significance Correction

PCT_Diff_Placebo_first

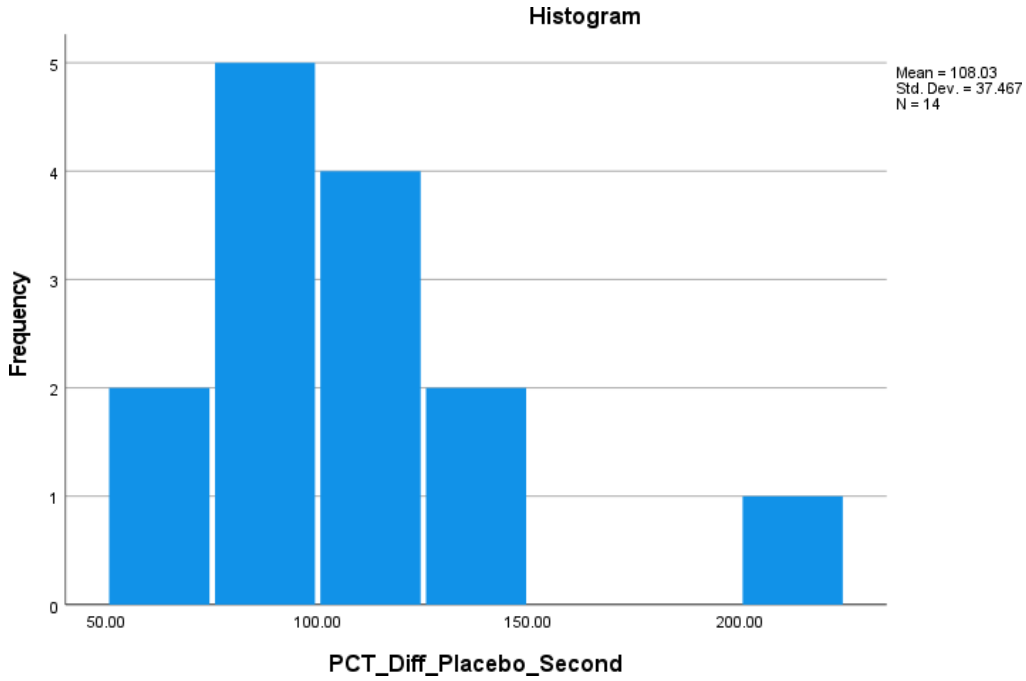

Histogram
Mean = 93.47
Std. Dev. = 26.82
N = 14
Frequency
0
2
4
6
25.00
50.00
75.00
100.00
125.00
150.00
PCT_Diff_Placebo_first

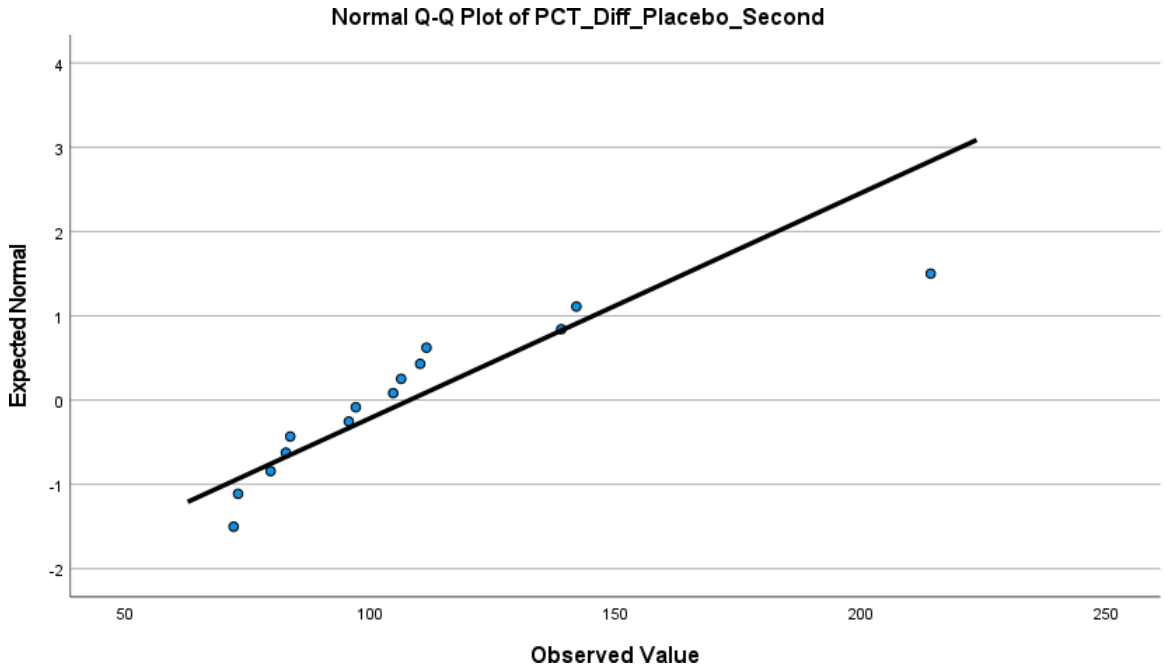

Normal Q-Q Plot of PCT_Diff_Placebo_first
Expected Normal
3
2
1
0
-1
-2
-3
50
100
150
Observed Value

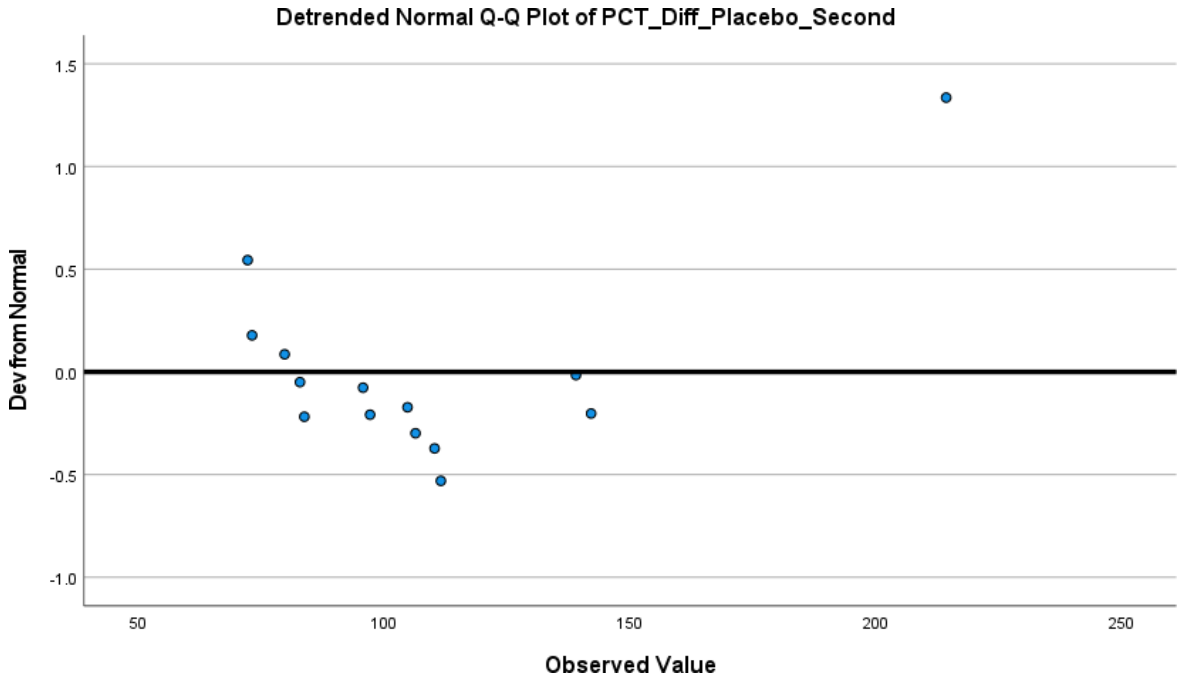

Detrended Normal Q-Q Plot of PCT_Diff_Placebo_first
Dev from Normal
0.75
0.50
0.25
0.00
-0.25
-0.50
25
50
75
100
125
150
Observed Value

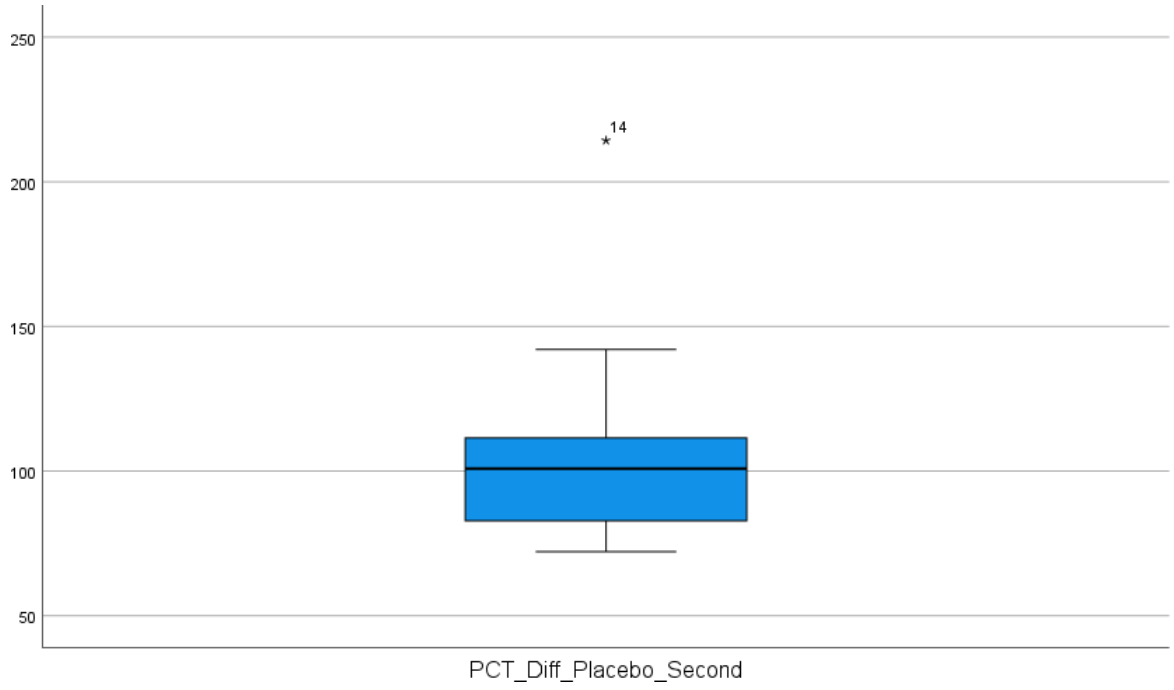

12
150
125
100
75
50
11
25
PCT_Diff_Placebo_first

PCT_Diff_Placebo_Second

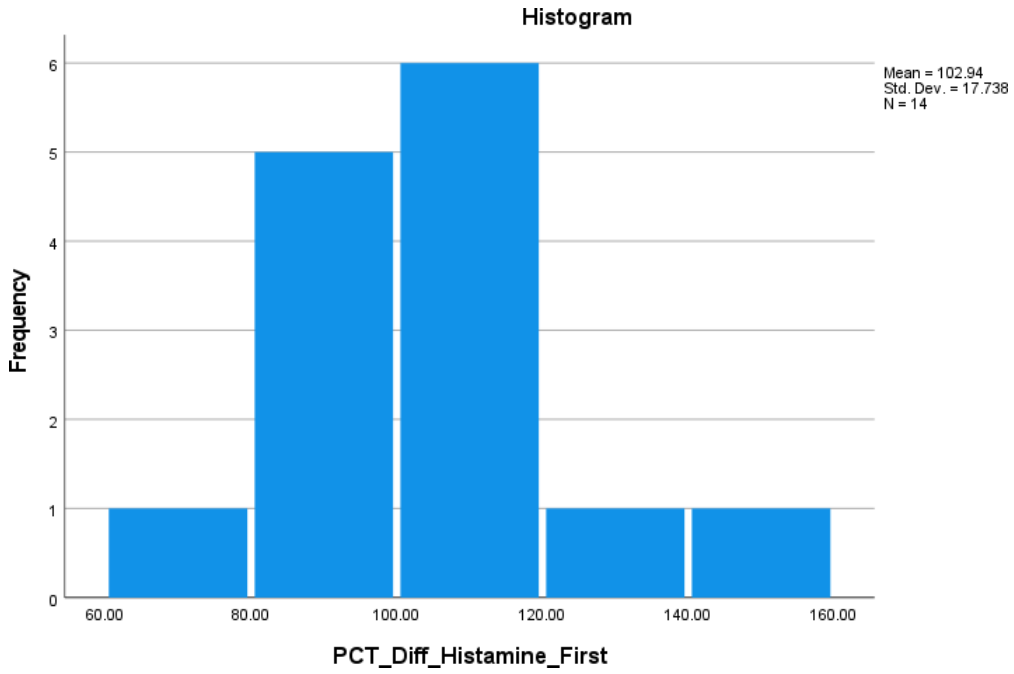


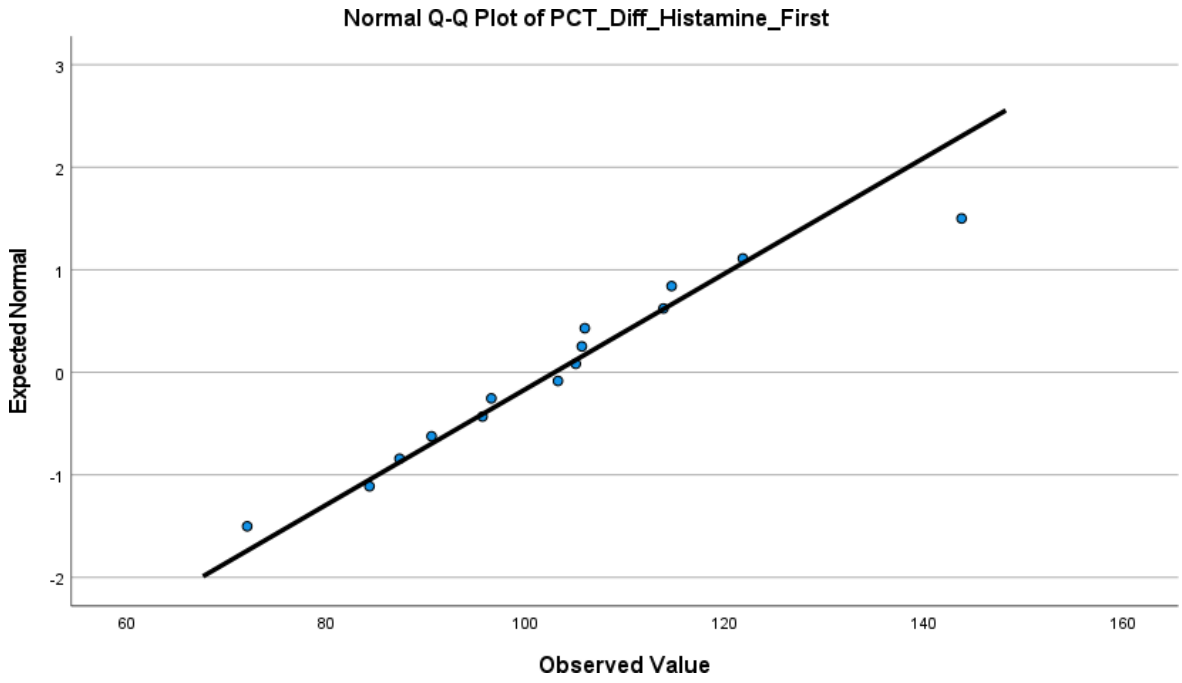


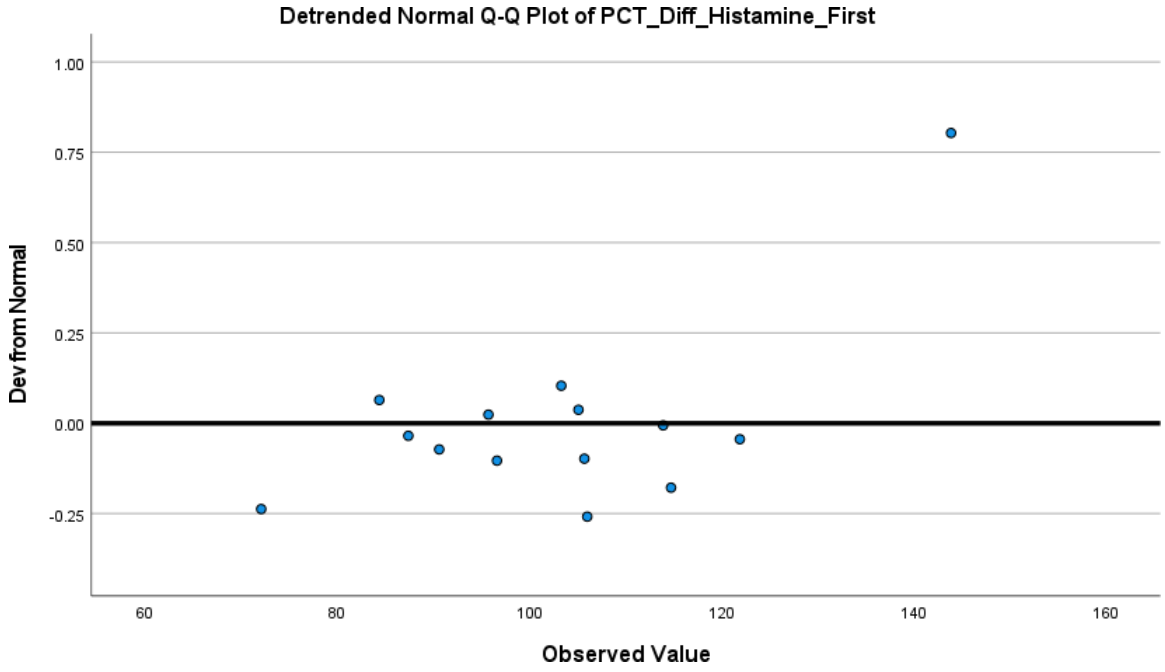

Detrended Normal Q-Q Plot of PCT_Diff_Placebo_Second
Dev from Normal
1.5
1.0
0.5
0.0
-0.5
-1.0
50
100
150
200
250
Observed Value

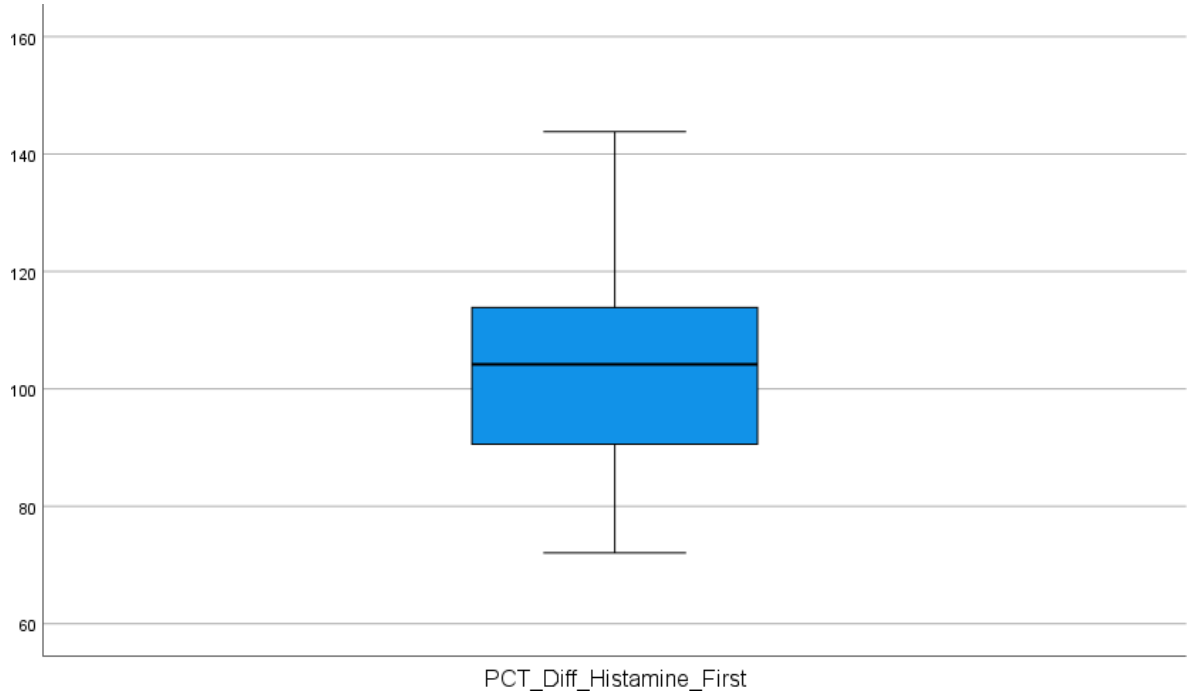

250
200
150
100
50
14
PCT_Diff_Placebo_Second

PCT_Diff_Histamine_First

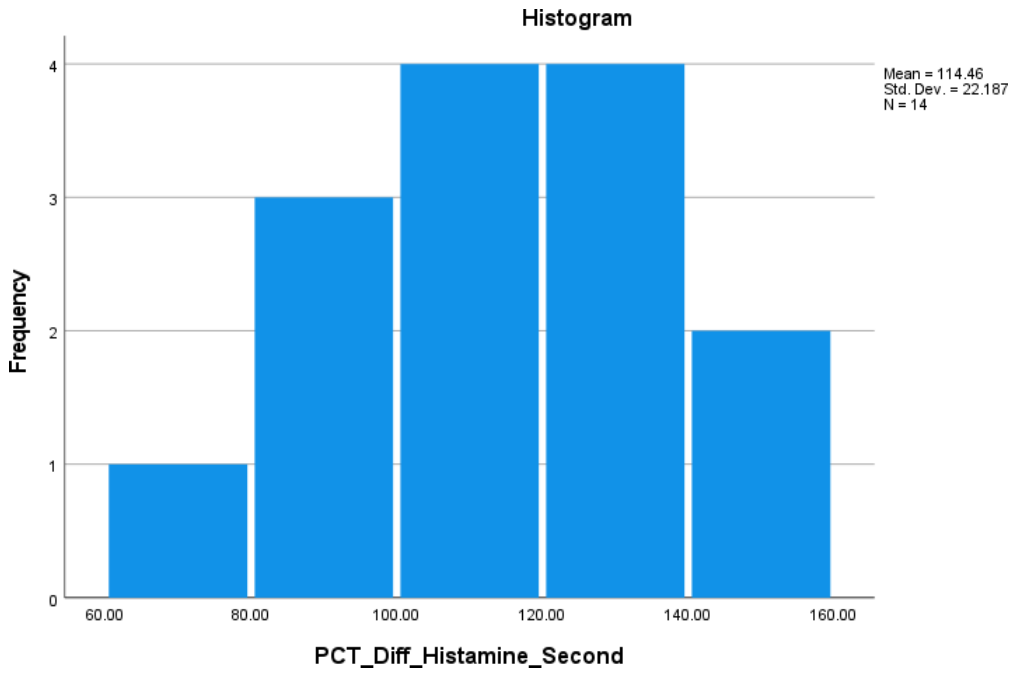


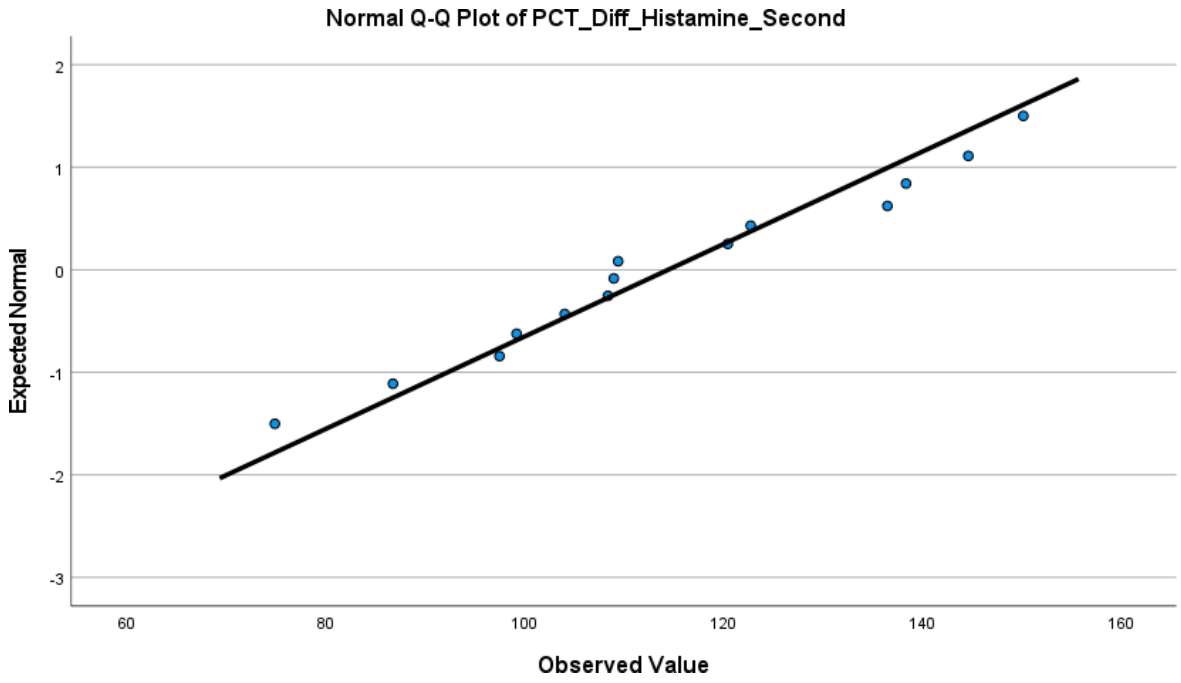

Normal Q-Q Plot of PCT_Diff_Histamine_First
Expected Normal
3
2
1
0
-1
-2
60
80
100
120
140
160
Observed Value

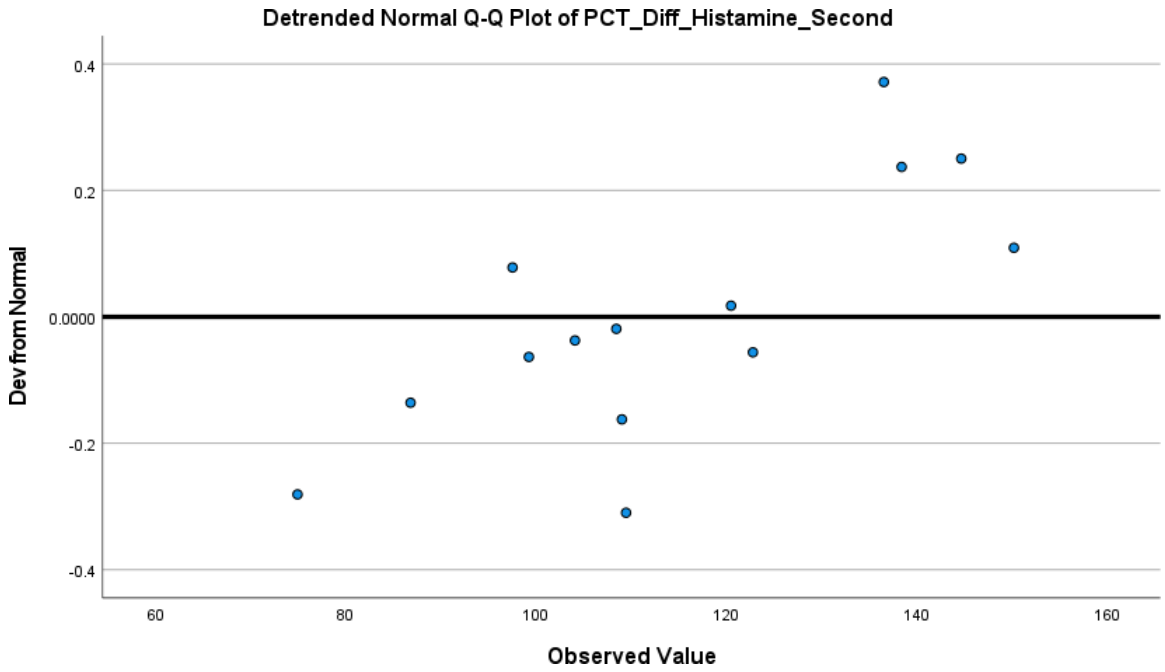

Detrended Normal Q-Q Plot of PCT_Diff_Histamine_First
Dev from Normal
1.00
0.75
0.50
0.25
0.00
-0.25
60
80
100
120
140
160
Observed Value

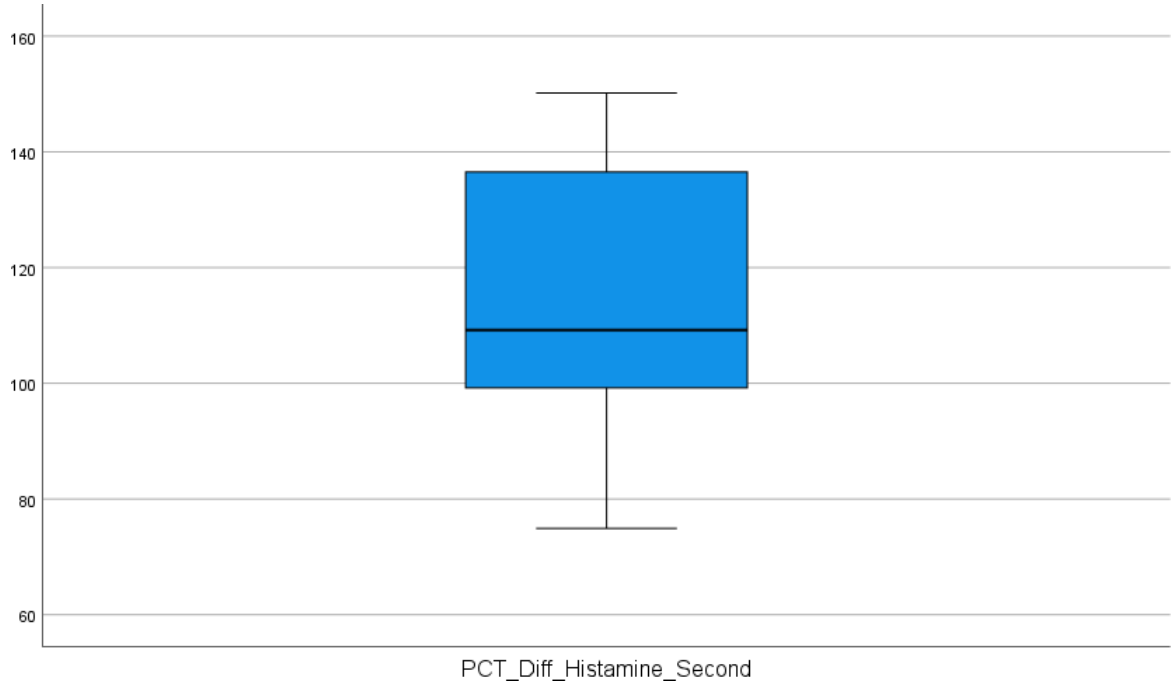


PCT_Diff_Histamine_Second




Normal Q-Q Plot of PCT_Diff_Histamine_Second
Expected Normal
2
1
0
-1
-2
-3
60
80
100
120
140
160
Observed Value


Detrended Normal Q-Q Plot of PCT_Diff_Histamine_Second
Dev from Normal
0.4
0.2
0.0000
-0.2
-0.4
60
80
100
120
140
160
Observed Value


160
140
120
100
80
60
PCT_Diff_Histamine_Second