Influences of Sound, Touch and a Combination of Both on Neuronal Activity in Cricket Legs

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SUMMARY:

For this final experiment, we wanted to investigate if tactile stimulation, when paired with sound, will increase neuronal activity when compared to only stimulation via solely sound or touch. We accomplished this through cricket leg stimulation. The problem we were trying to address is how the effect of stimulus-stimulus pairing impacts the neuronal activity, instead of just neuronal responses from a singular stimulus. Since we know that sensory receptors are triggered by certain stimuli, we hypothesized that touch and sound, when paired together, will produce different spiking activity compared to a cricket leg solely exposed to sound or touch. We predicted that sound and touch together will result in greater neuronal spiking than just exposing the cricket leg to solely sound or touch stimulus. For our methods, we wanted to replicate the experiment, so we used two different legs from two different crickets. We exposed both legs to the song "Sail" by AWOLNATION, then we exposed them to a consistently strong, constant poking (two second poking on with a toothpick, two seconds off), and a combination of the two—we also provided the legs with a full two minutes rest between each of the three trials. We measured the neuronal activity of the legs using the SpikeRecorder software on a Mac computer. We resulted that, on average, touch and sound alone caused more neuronal activity than the control and the combined stimulus, as the combined stimulus resulted in the lowest frequency. The control (with no stimulation) resulted in the second lowest frequency, while touch and sound were the highest and second highest frequency respectively. We chose to conduct these experiments because we are interested in possible therapeutic avenues which may benefit from the combination of stimuli—such as Autism therapeutic treatments of environmental enrichment, including sound paired with tactile stimulation and other stimulus-stimulus pairings (Lepper, T.L. & Petursdottir, A.I., 2017). In both cricket legs, the combined sound and touch stimulus had lower frequencies recorded than for sound and touch separate from each other, as well as from the control. These lower frequencies correlate to lower spike rates, which equate to decreased neuronal activity. This could be important because our results, if able to be replicated, could contribute to potential treatments for those struggling with sensory overload issues or those who get easily overstimulated.

INTRODUCTION/BACKGROUND/OBJECTIVES:

In previous studies in this class, we have observed neuronal responses to both sound and touch separately. In one lab (lab 3), we took a toothpick and stimulated the barbs on the tibia of a cricket leg using constant pressure and consistent reactions/poking (which was similar to our touch stimulus in this lab). We found that constant pressure gave consistent reactions from the spike trains, while repetitive poking makes the spike trains differentiated—spikes were higher depending on the poke force and one could see the separate pokes in the spike train quite clearly.

In the second part of lab 3, we found that a song with a lot of bass ("TikTok" by Ke$ha) lead to a lot of consistent twitching of the leg. In a song with a lot of treble (Bach's Violin Concerto No. 1), we observed the cricket leg to twitch more intensely and sporadically (with more complex spike trains). The gap in knowledge which we ventured to fill with this final lab was how neurons react to a combination of these two stimuli—namely sound (with a bass heavy song) and touch (with consistent poking).

Our main objective for studying the combination of two stimuli was to visualize neuronal responses of stimuli-stimuli pairing—this objective is due to previous studies, where stimulus-stimulus pairing has had some successes (Carroll, R. A., & Klatt, K.P., 2008), and some failures (Esch, B. E. et al., 2005), in treating various disorders such as Autism. Carroll and Klatt paired sound with a preferred stimulus picked by a child participant, in this case it was a toy. This aided in conditioning automatic reinforcement and helped increase vocalization in one of the participants. However, Esch, B. E. et al. found opposing results. They found that echoic responses from autistic children did not increase following their stimulus-stimulus pairings. Studying the neuronal responses from crickets from stimulus-stimulus pairing could illuminate potential therapeutic avenues for humans, as the spike trains could help us understand how human brains would react to similar stimulus-stimulus pairings.

The scientific purpose of this study is to examine neuronal responses to different stimulus—and, most importantly, to compare and contrast the neuronal responses to one singular stimulus with a combination of two stimuli in tandem. The hypothesis being tested is that touch and sound, when paired together, will produce different spiking activity compared to a cricket leg solely exposed to sound or touch. The experimental strategy's object was to provide an unbiased experimental set-up—we made sure to record controls from each leg, let the legs rest between trials, and repeat the experiment. This study system is appropriate to answer the hypothesis because it was unbiased; we utilized the same song and the same tactile stimulus (with the same strength and same timing) to both of the legs, with the same amount of rest as well.

METHODS/EXPERIMENTAL APPROACH:

To test this hypothesis, we ran three different experiments on two different cricket legs; we used what are known as "common crickets," or Acheta domesticus. We wanted to test how the cricket leg would respond to a sound stimulus, a touch stimulus, and then a combination of the two. To measure neuronal activity we used the SpikerBox and "SpikeRecorder" software. After the experiments were performed, we recorded, screenshotted and analyzed both the spike trains and the frequencies. We then plotted the data points and calculated the R^2 value to see the correlation in our data.

For recording the response to the touch stimulus, a needle electrode was inserted into the tibia and femur of the first cricket leg, as well as the cork on the SpikerBox (Fig. 1a). As for the method to apply the touch stimuli, we used a toothpick and applied pressure to the cricket leg using constant poking. For the sound stimulus, as well as the combined touch and stimulus, the needle electrodes were placed in the same locations as for touch (Fig. 1a), but a stimulation cable was plugged in to the headphone jack, and micro-clips from the stimulation cable were attached to the needle electrodes placed in the cricket leg (Fig. 1b).

The first cricket leg's neuronal activity was recorded without any stimulation to serve as a control. We then let the cricket leg rest for two 2 full minutes. Next, the leg was exposed to a sound stimulus. We used the song "Sail" by AWOLNATION because it has a high amount of bass and we received a significant response from the cricket leg from lab 3 when exposing the leg to a song that was bass heavy. The volume was turned all the way up and the activity of the cricket leg was recorded at the beginning of the song, and 20 seconds in, since the bass in these portions were very different. The cricket leg then rested for a full two minutes before being exposed to the tactile stimulation. Pressure applied using a toothpick was just enough to visibly push down the leg. The femur of the leg was poked for two seconds on, two seconds off, etc., for a total of six rounds before the touch stimulus was applied to the tibia using the same technique. Neuronal activity was recorded for both locations. After another two-minute rest, the cricket leg was exposed to its final experiment. The first cricket leg was exposed to a combination of sound and touch stimulation using the same song and poking method as described above, but this time simultaneously. Data was recorded for pressure applied to the femur at the start of the song at the same volume, as well as pressure applied to the tibia 20 seconds in.

We wanted to make sure we covered a range of bass in the song, as well as applying pressure to different positions on the leg, when taking our measurements to see the full effect of the different stimulations on the cricket legs. We thought that the bass at the start of the song was more choppy and less frequent, while the bass 20 seconds into the song was deeper and more consistent. We also wanted to apply touch stimulus to different areas of the leg, to make sure we saw how the entire leg was being affected. The point of the experiment was to compare touch and sound stimuli separate, and then combine them to see if the neuronal activity is additive, linear, or has minimal correlation. To achieve this, we thought it would be best to get a variety of readings and average how sound and touch, overall, affects the frequency.

The second cricket leg underwent the same procedure as the first cricket leg. Neuronal activity was measured at the same time, using the same song, volume, and poking techniques. Spike trains and frequencies for each experiment were recorded.

RESULTS:

The first cricket leg had a frequency of 322.972 Hz when no stimulation was applied (Fig. 2-4). This served as our control, giving us a baseline reading to compare the frequencies from the stimulation experiments. After a two-minute rest, the leg was then exposed to the sound stimulus. The recorded frequencies for the start of the song as well as 20 seconds in were 210.863 Hz and 578.491 Hz respectively (Fig. 2). This makes sense because the amount of bass, and other levels of sound, increases as the song proceeds. After the two-minute rest, the leg was exposed to the touch stimulation. When pressure was applied to the femur using the technique mentioned above, the frequency was measured at 555.972 Hz (Fig. 3). When pressure was applied to the tibia, the frequency was 529.359 Hz (Fig. 3). Next, a combination of sound and touch stimulus were applied to the cricket leg simultaneously. The frequency for the start of the song combined with pressure to the femur was 173.170 Hz (Fig. 4). The frequency for 20 seconds into the song with pressure applied to the tibia was 462.396 Hz(Fig. 4).

For the second cricket leg, the control reading was 377.712 Hz (Fig. 5-7). The sound stimulation for the start of the song was 190.929 Hz, while at 20 seconds we recorded a frequency of 555.056 Hz (Fig. 5). For the touch stimulation, pressure applied to the femur produced a frequency of 473.859 Hz, and pressure to the tibia produced a measurement of 496.274 Hz (Fig. 6). The combination of touch and sound produced a frequency of 201.193 Hz at the start of the song while pressure was applied to the femur, and 433.131 Hz 20 seconds into the song while pressure was applied to the tibia (Fig. 7). Using all these data points, we averaged the frequencies for each stimulation method to see how they impacted the neuronal activity of the entire leg (Fig. 8).

The experiments performed on both cricket legs proved our hypothesis to be true but disproved our prediction. We hypothesized that exposing the cricket leg to a combination of stimuli would produce a different spiking rate than the cricket leg when exposed to no stimulation. The alternative hypothesis would then be that exposing the cricket leg to a combination of stimuli would not produce a different spiking rate than the control. Our prediction was that the spiking rate from the combination of stimuli would be much greater than the control, and higher than any of the stimuli applied by themselves. We do see a different spiking rate from the stimulus pairing when compared to no stimulation, however, contrary to our prediction, the spiking rate decreased from the control (Fig. 8).

When comparing the data points between cricket legs, you can see that both legs generally follow the same pattern and have similar trendlines (Fig. 9). The R^2 values for both legs are relatively small. Cricket leg #1 has an R^2 value of 0.0181, and cricket leg #2 has an R^2 value of 0.0048 (Fig. 9). A low R^2 value means that there is high variability around the regression line, and therefore the type of stimulation does not explain the recorded frequencies. We had predicted that there would be more correlation between multiple stimulation methods and higher spike rates, or frequencies. This was not the case. Our results do not appear linear, nor additive (Fig. 8 and Fig. 9). In fact, the lowest average frequency measured was from the combined sound and touch stimuli at 317.473 Hz. The sound stimulus alone averaged at 383.835 Hz, while touch alone averaged at 513.855 Hz. However, our hypothesis was supported, because we did see a different spiking rate between no stimulation (averaged at 350.342 Hz) and the sound and touch stimuli combined (317.473 Hz).

SIGNIFICANCE/FUTURE WORK:

For this final experiment, we wanted to investigate if the paring of tactile stimulation with sound will increase neuronal activity when compared to only stimulation via solely sound or touch. Our hypothesis was that touch and sound together will produce different spiking activity compared to a cricket leg solely exposed to sound or touch, and our experiment was ample for testing this hypothesis. We thought this study was important to understand how pairing stimulations affects neuronal activity, as there has been some success with stimulus-stimulus pairing (Carroll, R. A., & Klatt, K.P., 2008). These successes could be promising for possible treatments for different neurological/developmental disorders.

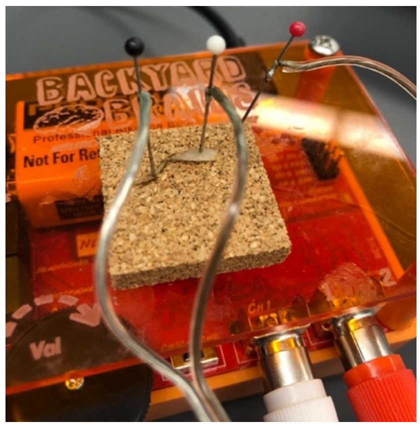
However, we concluded that, on average, touch and sound alone caused more neuronal activity than the control and the combined stimulus, as the combined stimulus resulted in the lowest frequency. The control resulted in the second lowest frequency, while touch and sound were highest. We chose to conduct these experiments because we are interested in possible therapeutic avenues which may benefit from the combination of stimuli—such as Autism therapeutic treatments of environmental enrichment, including sound paired with tactile stimulation and other stimulus-stimulus pairings (Carroll, R. A., & Klatt, K.P., 2008). Although our conclusions are in opposition to this (Carroll, R. A., & Klatt, K.P., 2008), we still believe that any data is helpful in these studies and might result in further studies to gain deeper understanding in how stimulus-stimulus pairing effects neuronal activity. For future experiments, we hope that our results could lead to more interest in stimulus-stimulus pairing studies. These lower frequencies that we found in our results from the combined sound and touch stimulus correlate to lower spike rates, which equate to decreased neuronal activity. Our results, if able to be replicated, may point towards a potential treatment for those struggling with sensory overload issues and overstimulation.

One weakness of our study, and a plausible reason why we did not see the results we hoped for, was that there was a lot of background noise from other experiments going on in the same room. If we were to replicate this experiment, ideally, we would conduct it in a more controlled environment with less interference. Increasing our sample size to more than just two cricket legs could also strengthen the results of our experiment. Also, sound and touch may be too similar to compare. The auditory information from the song could be affecting the bending of the sensory hairs without the touch stimulus being applied. Given this information, we would also consider testing different sensory modalities, such as smell or vision, to see how a combination of those stimuli effect neuronal spiking activity.

This experiment elucidated further avenues of research that one could pursue from the results. Even though our prediction was not supported, the data collected is still valuable for future experiments.

Figure 1: (a) The experimental set up for the touch stimulus. The white needle electrode is placed in the femur of the cricket leg while the black need electrode is placed in the tibia. The red needle electrode is inserted into the cork board. (b) The experimental set up for the sound and touch/sound combined stimulus. Needle electrode placement same as in (a). A stimulation cable was plugged in to the headphone jack, and micro-clips from the stimulation cable were attached to the needle electrodes placed in the cricket leg.

a.



b.

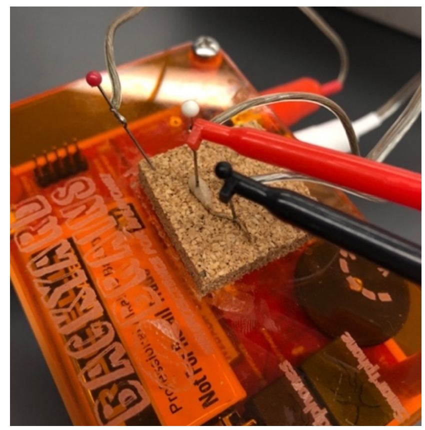


Figure 2: Stimulation via sound for cricket leg #1. The song, volume, as well as the time of the song when the measurement was taken is listed along with their corresponding spike trains and frequencies.

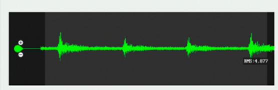
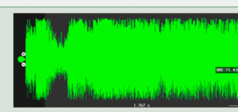
Stimulation Method	Spike Train	Frequency
No stimulation (control)		322.972 Hz No physical movement of the leg
Sound: "Sail" by AWOLNATION Volume: max At the start of the song		210.863 Hz No physical movement of the leg
Sound: "Sail" by AWOLNATION Volume: max 20 seconds into the song		578.491 Hz No physical movement of the leg

Figure 3: Stimulation via touch for cricket leg #1. Stimulation was applied by poking the designated area of the leg with a toothpick for two seconds on, then two seconds off, etc. The area of the leg the pressure was applied to as well as its corresponding spike trains and frequencies are shown here.

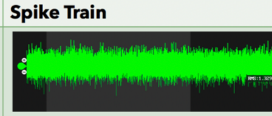
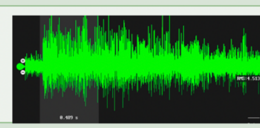
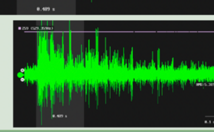
Stimulation Method	Spike Train	Frequency
No stimulation (control)		322.972 Hz No physical movement of the leg
Constant Poking (2 seconds on, 2 seconds off) Applying pressure to the femur		555.929 Hz
Constant Poking (2 seconds on, 2 seconds off) Applying pressure to the tibia		529.359 Hz

Figure 4: Stimulation via sound and touch simultaneously for cricket leg #1. Sound stimulus method same as fig. 2, and touch stimulus method same as fig. 3. Spike trains and frequencies were recorded for the different segments of the song, as well as the different areas pressure was applied to.

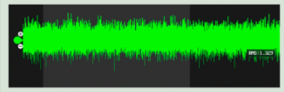
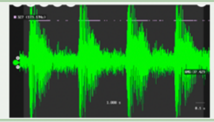
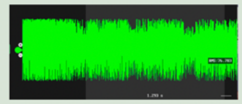
Stimulation Method	Spike Train	Frequency
No stimulation (control)		322.972 Hz No physical movement of the leg
Sound: "Sail" by AWOLNATION Volume: max Applying pressure to the femur		173.170 Hz
Sound: "Sail" by AWOLNATION Volume: max Applying pressure to the tibia		462.396 Hz

Figure 5: Stimulation via sound for cricket leg #2. The song, volume, as well as the time of the song when the measurement was taken is listed along with their corresponding spike trains and frequencies.

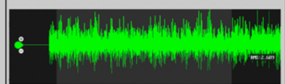
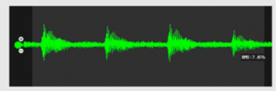
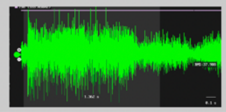
Stimulation Method	Spike Train	Frequency
No stimulation (control)		377.712 Hz No physical movement of the leg
Sound: "Sail" by AWOLNATION Volume: max At the start of the song		190.929 Hz No physical movement of the leg
Sound: "Sail" by AWOLNATION Volume: max 20 seconds into the song		555.056 Hz No physical movement of the leg

Figure 6: Stimulation via touch for cricket leg #2. Stimulation was applied by poking the designated area of the leg with a toothpick for two seconds on, then two seconds off, etc. The area of the leg the pressure was applied to as well as its corresponding spike trains and frequencies are shown here.

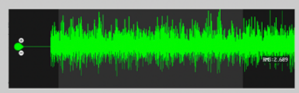
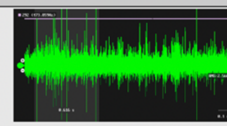
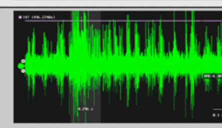
Stimulation Method	Spike Train	Frequency
No stimulation (control)		377.712 Hz No physical movement of the leg
Constant Poking (2 seconds on, 2 seconds off) Applying pressure to the femur		473.859 Hz
Constant Poking (2 seconds on, 2 seconds off) Applying pressure to the tibia		496.274 Hz

Figure 7: Stimulation via sound and touch simultaneously for cricket leg #2. Sound stimulus method same as fig. 5, and touch stimulus method same as fig. 6. Spike trains and frequencies were recorded for the different segments of the song, as well as the different areas pressure was applied to.

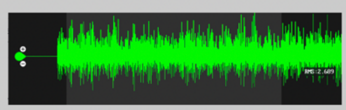
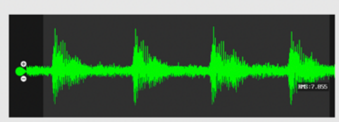
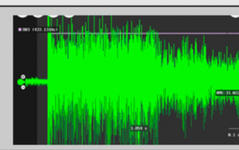
Stimulation Method	Spike Train	Frequency
No stimulation (control)		377.712 Hz No physical movement of the leg
Sound: "Sail" by AWOLNATION Volume: max Applying pressure to the femur		201.193 Hz
Sound: "Sail" by AWOLNATION Volume: max Applying pressure to the tibia		433.131 Hz

Figure 8: The data displayed here represents the averages of the trials from both cricket legs. The control (blue) is the average of both control frequencies recorded. The sound stimulus (grey) is the average of the sound stimulus from the start of the song and 20 seconds. The touch stimulus (light blue) is the average of the frequencies from pressure applied to the femur and tibia. The sound and touch stimulus bar (navy blue) is the average of the frequencies measured from the start of the song while poking the femur, and 20 seconds into the song while poking the tibia.

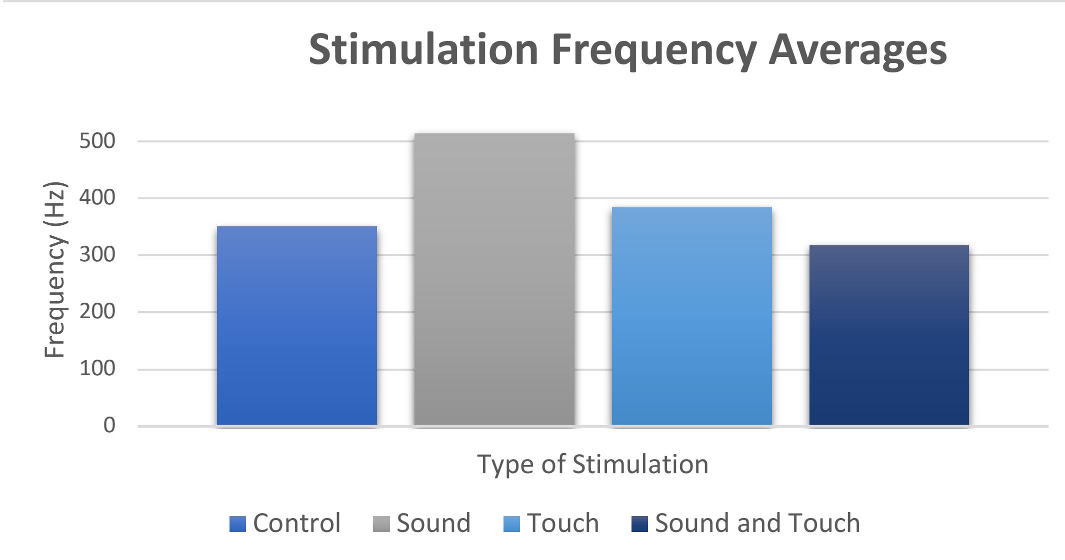
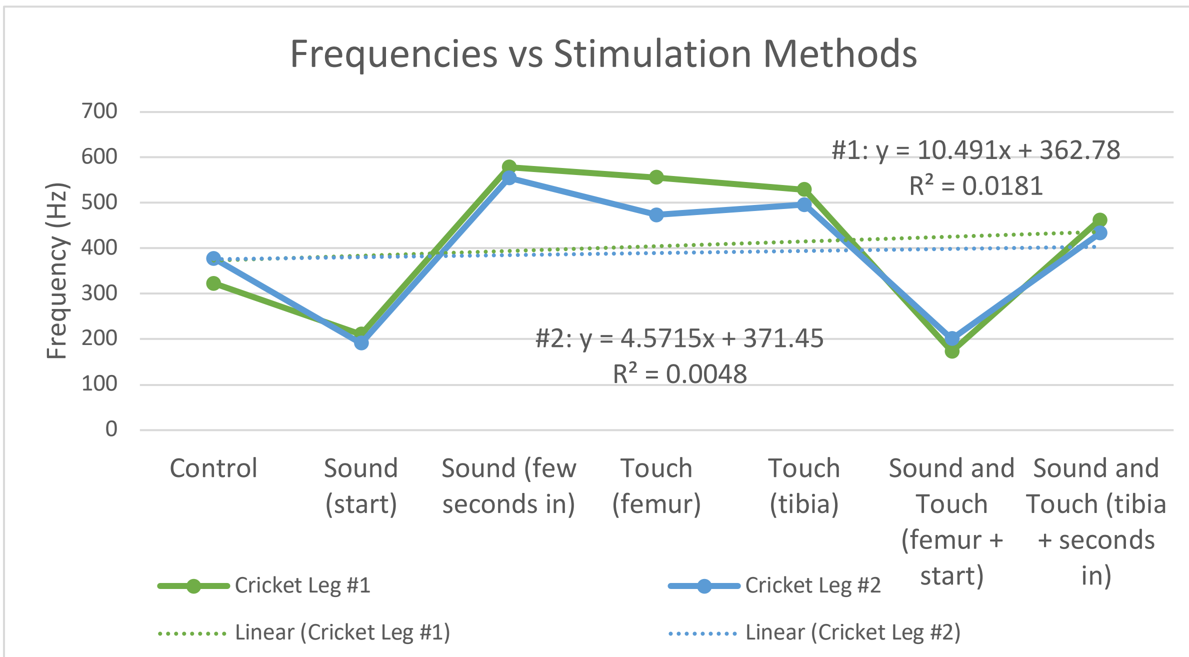


Figure 9: Different stimulation methods and their corresponding frequencies for cricket leg #1 (solid green) and cricket leg #2 (solid blue). The dashed lines represent the trendline for the cricket legs, and the R^2 value as well as the equation for both lines are presented.



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Esch, B. E., Carr, J. E., & Michael, J. (2005). Evaluating stimulus-stimulus pairing and direct reinforcement in the establishment of an echoic repertoire of children diagnosed with autism. The Analysis of verbal behavior, 21(1), 43–58.

Lepper, T.L. and Petursdottir, A.I. (2017), Effects of response-contingent stimulus pairing on vocalizations of nonverbal children with autism. Jnl of Applied Behav Analysis, 50: 756-774. https://doi.org/10.1002/jaba.415