The observation of contingent negative variation with reaction times in non-human primates

By

Nam Soo Kim

Thesis

Submitted to the Faculty of the

Graduate School of Vanderbilt University

in Partial fulfillment of the requirements for

the degree of

Master of Arts

In

Psychology

May, 2013

Nashville, Tennessee

Approved:

Professor Geoffrey F. Woodman

Professor Jeffrey D. Schall

Abstract

A half-century's worth of research has established the existence of numerous event-related potential (ERP) components measuring different covert cognitive operations in humans including preparing responses to anticipated events. An ERP component referred to as contingent negative variation (CNV) is believed to be an index of movement preparation and initiation during the interval between a warning stimulus and an imperative stimulus triggering a response. Using an electrophysiological technique analogous to procedures for recording scalp ERPs from humans, we show that macaque monkeys exhibit a CNV during a memory-guided saccade task. First of all, we observed the highest amplitude of CNV over the motor cortex. Second, the amplitude in CNV was related to the reaction times. Thus, we conclude that CNV observed in non-human primates like CNV observed in humans is a covert mechanism underlying the preparation and initiation of voluntary movements.

Introduction

Before making a planned action, all living creatures are ready and prepare the onset of go signal. It makes sense that the more prepared, the faster reaction. However, The relationship between motor preparation and reaction times has still been remained unclear. Behavioral studies showed how motor preparation modulated reaction times (Luce, 1986; Niemi & Näätänen, 1981). However, only behavioral method is not enough to investigate covert processes between motor preparation and initiation. During past years, single cell recording studies has tried to elucidate how the preparatory activity preceding movement influence the determination of where and when to move the eyes (Schall & Thompson, 1999; Shadlen & Newsome, 2001). At single neuron level of saccadic system, moreover, a specific link between motor behavior and activation neuron was clearly elucidated (Hanes & Schall, 1996). However, recent study has showed that the neuronal activity at the population level than a single neuron level was highly correlated with preparatory phase (Churchland, Cunningham, Kaufman, Ryu, & Shenoy, 2010). This recent research gives rise to need about how we can study the neuronal activity of population level during preparatory phase.

A candidate of event-related potentials (ERPs) underlying motor preparation is contingent negative variation (CNV). CNV is characterized by a negative going activity built up during motor preparation period between 'ready' and 'go' signals (Walter, Cooper, Aldridge, McCallum, & Winter, 1964) and has observed over the motor cortex (Ng, Tobin, & Penney, 2011). Also, CNV highly appeared over the motor cortex during the task related to either eye or hand (Verleger, Wauschkuhn, van, Jaśkowski, & Trillenberg, 2000). Moreover, it is clear that the amplitude in CNV is highly correlated with the reaction times (Trillenberg, Verleger, Wascher, Wauschkuhn, & Wessel, 2000). These findings implicate that CNV would be the neuronal activity representing the crowed of neurons during preparatory phase.

However, The fact that there are a few studies about CNV from non-human primates is not reasonable because a bunch of evidence has been shown that the intracranial neuronal activity at single neuron level from non-human primates has been highly related to reaction times. Thus, the evidence implicates that an extracranial neuronal activity at population level will also show close relation with reaction times. Thus, this study tried to investigate whether CNV might be not only observed in non-human primates but also related to reaction times.

We trained the animals to perform memory guided saccade task in which the delay period, preparation phase, between ready and go signals ranged from 0 to 1000 ms. First of all, we found the CNV from non-human primates shows the highest amplitude at Cz located on the motor cortex in 10-20 systems. Second, we found that the faster reaction times, the more negative CNV amplitude. Taken together, we dare to conclude not only that like CNV recorded from humans, the CNV also appeared in non-human primates during preparatory phase but also that the CNV was highly related to reaction times.

Methods

Subjects

Data were collected from monkey B and X (male and female rhesus monkeys (Macaca mulatta), the animals were cared for in accordance with policies set forth by the USDA and Public Health Service Policy on Humane Care and Use of Laboratory Animals. All procedures were performed with supervision and approval from the Vanderbilt Institutional Animal Care and Use Committee. All surgical procedure were carried out under aseptic conditions. The animals were anesthetized with ketamine (15 mg / kg) and buprenex (0.01 mg/kg) before intubation and catheterization. The head was shaved and scrubbed with betadine and 70 % alcohol after mounting in a stereotaxic device. The

animals were anaesthetized with isoflurane (2%). ECG, rectal temperature, and respiration were monitored. A stainless steel post was attached to the skull to restrain the head during experiment. Five (for monkey B) and four (for monkey X) gold-plated electrodes were implanted in the skull surface. The Teflon-coated multistranded stainless steel leads from each electrode were collected in one plastic connector that was embedded in acrylic cement bonded to the headpost. The impedance of the EEG electrodes once implanted was 2 - 5 kΩ measured at 30 Hz.

Behavioral procedure

The animals were seated in enclosed primate chairs and head restrained using surgically implanted headpost. Stimulus presentation and delivery of liquid reward were all under computer control in hard real time (TEMPO, Reflective Computing, Olympia, WA). Visual stimuli were presented using computer-controller raster graphics (TEMPO Videosync 640 x 400 pixel resolution, Reflective Computing, Olympia, WA). Stimulus sizes were auto-calculated by the computer program to account for subject viewing distance. Their luminance values were 10 cd/m2 on a 0.02 cd/m2 background.

The animals were trained for memory guided saccade task. All trials began with the presentation of central filled white square (0.5°). They were required to fixate the central square for various fixation delay period (350 - 450 ms for monkey B and X). After the delay, peripheral white square (3°) target briefly (14 ms) appeared at two possible locations (left and right) of 10° eccentricity on the horizontal meridian. The animals were required to maintain fixation at the central spot for a delay period (0 – 1000 ms). After the delay period had elapsed, they generated a saccade to the location where the target had appeared (within 1000 ms). They were rewarded if making a saccade to the location that the target had appeared.

Data acquisition

Implanted surface electrodes were referenced to link both ears using ear-clip electrodes (Electro-Cap International). All electrode impedances were less than 10 kΩ. The signals from each electrode was amplified 1k times with a high input impedance head stage (Plexon) and filtered between 0.7 and 170 Hz (bandpass filter). The EEG signal was collected from five surface electrodes (Fz, Fcz, Cz, O1, and O2) for monkey B, and from four surface electrodes (Fz, F3, F4, and Cz) for monkey X. Eye position was tracked using an infrared eye tracking system (Applied Science Laboratories). Horizontal and vertical eye positions were calibrated, acquired, and streamed to the Plexon computer using the EyeLink 1000 infrared eye-tracking system with a resolution of 0.01° (SR Research Kanata, Ontario, Canada).

Data analysis

All reaction times calculated from the presentation of go signal. Trials with premature (before the go signal or < 70 ms) and express (< 150 ms), wrong and missing responses were excluded. Moreover, EEGs were digitally filtered with a zero phase shift 35 Hz low-pass hamming window (SD = 6 ms). And then EEG amplitude over 150 uV) were discarded.

For CNV analysis, all artifact free and correct response trials were chosen and then EEGs were aligned by the onset of target (ready signal) and truncated by 50 ms before the offset of fixation (go signal). Baselines were used from 150 and 50 ms before the onset of target. After that, each EEG was filed up and averaged across delay periods. This method is the same as the method by Trillenberg et al (Trillenberg et al., 2000). It makes sense because the animals did not know when the go signal came up.

For significant ERP differences, we used the method of difference between ERPs (Godlove et al., 2011). This method tests for differences in ERPs at between Fz and Cz

and in ERPs between faster and slower reaction times using a thresholding approach similar to those often used in single unit studies measuring activity onsets in spike-density functions. First, difference wave was calculated by subtracting ERPs in a condition from those in another condition. Negative difference wave values indicated that ERPs in a condition were more negative than ERPs in another condition, while positive difference wave values indicated an opposite polarity effect. Difference wave values near zero indicated no differences in ERPs between conditions. Thus, significant periods were defined as periods when the difference wave deviated from baseline by > 2 standard deviations (SDs) for longer than 50 ms, provided it exceeded 3 SDs in that interval.

Results

The characteristics of contingent negative variation

Contingent negative variation (CNV) is characterized by the negative going activity built up during preparatory phase. Verleger et al showed that the amplitude in CNV was the highest at the Cz regardless of eye- and movement-evoked CNV (Verleger et al., 2000). Thus, we also compared CNV at Cz with CNV at Fz. Figure 1 shows that the negative going activity built up during preparatory phase appeared after visual-evoked potentials. To compare CNV at Fz with CNV at Cz, we used the method that tests for differences between Fz and Cz ERPs using a threshold (Godlove et al., 2011). We found that the amplitude of visual-evoked potentials was significantly larger for at Fz than Cz (154 – 269 ms after the onset of target for monkey B; 37 – 263 ms after the onset of target for monkey X). However, the amplitude in the negative going activity built up during preparatory phase was significantly larger for at Cz than Fz (onset latency 302 ms after the onset of target for B and 317 ms for monkey X). Thus, These findings have two implications: first of all, consistent with human evidence, the CNV largely appear at Cz

even though eye-evoked CNV. The second is that the CNV could be well observed at least 350 ms after the onset of target.

Contingent negative variation with reaction times

Motor preparation and reaction times are closely related each other. Thus, we investigated whether the CNV was related to the reaction times. For the analysis, we used different delay periods between CNV and reaction times because the neural activity is modulated preceding the movement. Thus, delay periods were ranged from 350 to 950 ms for CNV and from 400 to 1000 ms for reaction times, respectively. First of all, the reaction times as function of delay periods are shown in Fig 2A. There were no significant differences in reaction times with elapsed delay period (For monkey X: $F(12,247) = 1.56$, $p > 0.10$; For monkey B: $F(12,182) = 0.53$, $p > 0.89$). It means that the motor preparation was constant across delay periods. However, a question about the relationship between the motor preparation and the variability of reaction times was still remained. The variability of reaction times that the animal made fast or slow is shown Fig 2B. Thus, we hypothesized that CNV underlying motor preparation might be different between faster and slow reaction times. However, two animals showed a significant difference in reaction times ($t(33) = 5.67$, $p < 0.001$). Thus, we split reaction times by the median of reaction times at each delay period for each animal. We found there were significant differences in the CNV between faster and slower reaction times. Figure 3 shows that the CNVs were more negative for faster than slower reaction times in both animals. For monkey X, the more negative activity for faster reaction times appeared during 309 to 452 ms and 508 to 751 ms from the onset of target. In contrast, the more positive activity for faster reaction times appeared during 222 to 301 ms from the onset of target. For monkey B, the more negative activity for faster negative activity was observed during 495 to 568 ms and 575 to 658 ms and 673 to 729 ms from the onset of

target. A large fluctuation after 800 ms from the onset of target might be caused by the small number of trials.

This finding implicates that the more negative indicated that the more neurons in the motor cortex involve in the preparation of movement. This is consistent with human evidence (Hillyard, 1969; Trillenberg et al., 2000). Thus, the CNV recorded from non-human primates reflects the covert process underlying motor preparation.

Discussion

The characteristics of contingent negative variation

CNV is defined as a negative going activity built up during preparatory phase between ready and go signals. A bunch of evidence showed that the amplitude of CNV was the largest at Cz located on the motor cortex (Ng et al., 2011; Trillenberg et al., 2000; Verleger et al., 2000). It implicates that the motor cortex involves in the preparation of movement. Thus, we hypothesized that CNV from non-human primates may also show the largest amplitude at Cz because humans and non-human primates share covert processes underlying the regulation of movement. We looked over CNV at Fz and Cz in both animals and found that like human CNV, the amplitude of CNV was the largest at Cz. This finding is consistent with previous evidence mentioned before. Thus, we claim that the CNV from non-human primates is the same as CNV from humans.

The relationship between CNV and reaction times

This study replicated previous evidence that the faster reaction times, the more negative CNV. It means that reaction times can be decreased as more neurons involve in the preparation of movement. In this study, the CNV responsible for motor preparation was more negative for faster than for slower reaction times. Thus, it is clear that the CNV from non-human primates is also related to reaction times.

However, in spite of this clear finings, there is a limitation about the relationship between the motor preparation and the estimation of timing of go signal. Shadlen et al showed the activity of neurons in the parietal cortex was modulated by the estimation of timing of upcoming go signal (Leon & Shadlen, 2003). Also, neurons in the premotor cortex coded the estimation of time (Mita, Mushiake, Shima, Matsuzaka, & Tanji, 2009). Likewise, CNV also reflected the expectation of the onset of go signal (Trillenberg et al., 2000). Moreover, CNV was modulated by the estimating time duration task in which the subject was required to judge the duration of a signal as being equal or not to that of a target (Macar & Vidal, 2003).

However, in this study, the reaction times were not gradually decreased as delay period increased. The first possible reason is that the monkey failed to inhibit making a saccade at longer delay period. As a result, the distribution of delay period was not the same as the rectangular distribution. Rather, the distribution was the similar as the exponential distribution. The posterior probability of exponential distribution is constant regardless of delay periods. The second reason is that we did not make any modulation of anticipation of go signal. Thus, the animal did not need to make a saccade as fast as possible or estimate a timing of go signal. Instead, only the state of whether prepared or not was reflected into CNV.

Nonetheless, we verified the existence of CNV in non-human primates. The amplitude in CNV was related to the reaction times. Numerous human studies have shown that CNV is an index of ERP component responsible for motor preparation. Countless studies have shown movement-related neurons in non-human primates activated during preparatory phase. However, there is a gap between humans and non-human primates evidence. Thus, this study is a demonstration that humans and non-humans share the mechanisms underlying the preparation and initiation of voluntary movement.

References

Churchland, M. M., Cunningham, J. P., Kaufman, M. T., Ryu, S. I., & Shenoy, K. V. (2010). Cortical preparatory activity: representation of movement or first cog in a dynamical machine? *Neuron, 68*(3), 387-400.

Godlove, D. C., Emeric, E. E., Segovis, C. M., Young, M. S., Schall, J. D., & Woodman, G. F. (2011). Event-related potentials elicited by errors during the stop-signal task. I. Macaque monkeys. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 31*(44), 15640-15649.

Hanes, D. P., & Schall, J. D. (1996). Neural control of voluntary movement initiation. *Science, 274*(5286), 427-430.

Hillyard, S. A. (1969). Relationships between Contingent Negative Variation (Cnv) and Reaction Time. *Physiology & Behavior, 4*(3), 351-&.

Leon, M. I., & Shadlen, M. N. (2003). Representation of time by neurons in the posterior parietal cortex of the macaque. *Neuron, 38*(2), 317-327.

Luce, R. D. (1986). Response Times: Their Role in Inferring Elementary Mental Organization. *New York: Oxford University Press*.

Macar, F., & Vidal, F. (2003). The CNV peak: an index of decision making and temporal memory. *Psychophysiology, 40*(6), 950-954.

Mita, A., Mushiake, H., Shima, K., Matsuzaka, Y., & Tanji, J. (2009). Interval time coding by neurons in the presupplementary and supplementary motor areas. *Nat Neurosci, 12*(4), 502-507.

Ng, K. K., Tobin, S., & Penney, T. B. (2011). Temporal accumulation and decision processes in the duration bisection task revealed by contingent negative variation. *Frontiers in integrative neuroscience, 5*, 77.

Niemi, P., & Näätänen, R. (1981). Foreperiod and simple reaction time. *Psychological Bulletin, 89*(1), 133-162.

Schall, J. D., & Thompson, K. G. (1999). Neural selection and control of visually guided eye movements. *Annual review of neuroscience, 22*, 241-259.

Shadlen, M. N., & Newsome, W. T. (2001). Neural basis of a perceptual decision in the parietal cortex (area LIP) of the rhesus monkey. *Journal of neurophysiology, 86*(4), 1916-1936.

Trillenberg, P., Verleger, R., Wascher, E., Wauschkuhn, B., & Wessel, K. (2000). CNV and temporal uncertainty with 'ageing' and 'non-ageing' S1-S2 intervals. *Clin Neurophysiol, 111*(7), 1216-1226.

Verleger, R., Wauschkuhn, B., van, d. L., Jaśkowski, P., & Trillenberg, P. (2000). Posterior and anterior contribution of hand-movement preparation to late CNV. *Journal of Psychophysiology, 14*(2), 69-86.

Walter, W. G., Cooper, R., Aldridge, V. J., McCallum, W. C., & Winter, A. L. (1964). Contingent Negative Variation: An Electric Sign of Sensorimotor Association and Expectancy in the Human Brain. *Nature, 203*, 380-384.

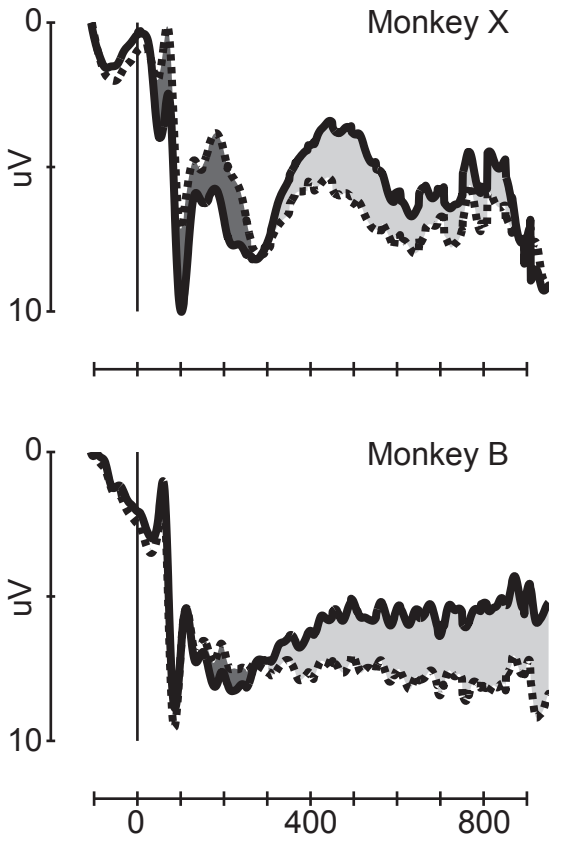

Figure 1. CNV at Fz (dashed line) and Cz (solid line) during memory guided saccade task. ERPs aligned by the onset of target are displayed. Vertical solid line indicates the onset of target. Dark gray areas indicate that the amplitude is significantly larger for at Fz than Cz. In constrast, Light gray areas indicate that the amplitude is significanly larger for at Cz than Fz.

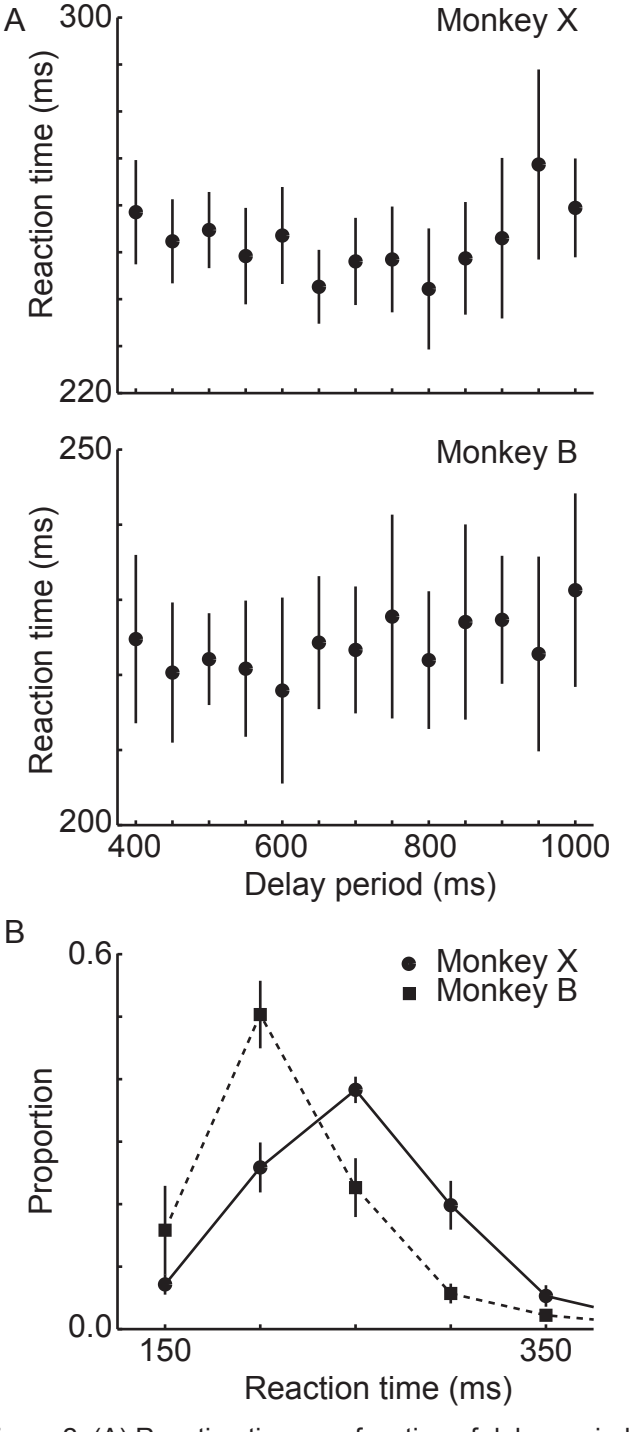

Figure 2. (A) Reaction times as function of delay period. Both animals shows constant reaction times with elasped delay period. (B) The distribution of reaction times between the animals. The proportion after 350 ms delay period is close to zero. The reaction times are faster for monkey B (filled square) than monkey X (filled circle). Vertical solid lines indicate 95 % confidence.

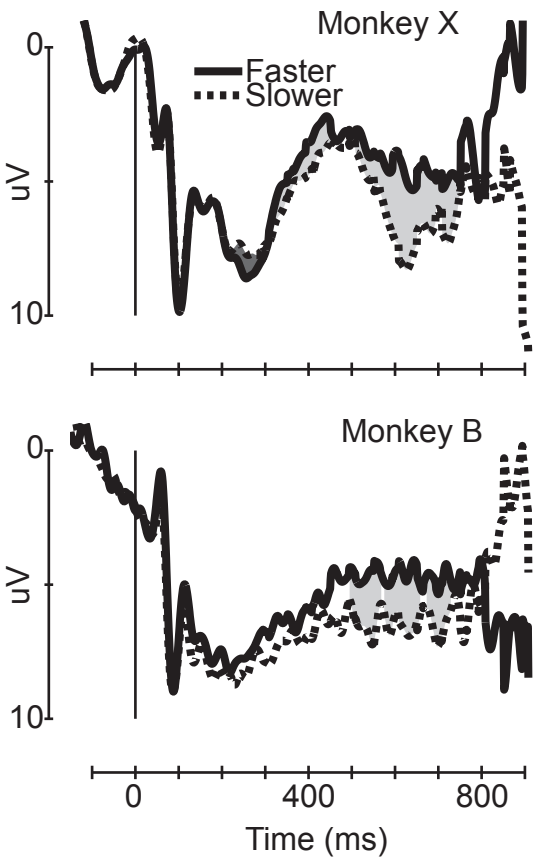

Figure 3. CNV between faster (solid line) and slower (dashed line) reaction times. The more negative, the faster reaction times. Grey areas indicate CNV is more negative for faster than slower reaction times. In constrast, Dark grey areas indicate CNV is more positive for faster than slower reaction times. Vertical lines indicates the onset of target.