including excised heart tissue, supports this[15–19]. One simple way to analyse such a system for linearity is to explore the functional dependence of the shape of one beat on the previous beat. We have generated 'scatter diagrams' (Fig. 2) of the T-wave peak height or T-wave area for the $N+1$st beat against the Nth. The degree of 'scatter' (range of ordinates corresponding to one abscissa) is a measure of how closely the $N+1$st beat depends for its shape solely on the shape of the Nth. Our plots show both the strong causal link between the Nth T-wave amplitude and the $N+1$st (that is, a small degree of scatter) and the nonlinearity of that causal relationship.

Further, from Li and Yorke's proof[20] the existence of tripling indicates that the cardiac system is sufficiently nonlinear to produce responses to atrial driving of all periodicities plus pseudostochastic sequences of waveforms. This leads us to ask why we saw no orders of period multupling higher than that of quintupling. A related question is why signals of a relatively low order of multupling alternate chronologically in our records (corresponding to similar heart rates and other system parameters) with aperiodic stretches. One answer is that there are several scenarios for the period multupling of noiseless nonlinear oscillators which predict the existence of intercalated bands of chaotic and periodic activity; in particular, the phenomenon of 'intermittency' is relevant[21,22]. Alternatively, noise disrupts the bifurcation structure of an idealized, non-linear system[8,9]. Higher order orbits are empirically more fragile (due to their smaller parameter space) and subject to effacement, so they are simply less likely to be observed. For example, Duffing's oscillator, when subjected to white noise, is found to develop a symmetrical gap in its bifurcation sequence. Higher-order orbits can even be induced solely by increasing the amplitude of the noise. In the heart, there are many sources of noise, in the form of stochastically varying parameters governing cardiac function; such as intrinsic fluctuations in the atrial rate, and variation in conduction and contractility due to local areas of ischaemia that develop in the stressed heart.

Our results support the model of the noradrenaline intoxicated heart as a highly nonlinear, driven oscillator subject to noise thus usefully linking many seemingly disparate dysrhythmias as bifurcative responses to variation in system parameters.

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Brain potentials during reading reflect word expectancy and semantic association

Marta Kutas & Steven A. Hillyard

Department of Neurosciences, M-008, University of California, La Jolla, California 92093, USA

The neuroelectric activity of the human brain that accompanies linguistic processing can be studied through recordings of event-related potentials (e.r.p. components) from the scalp. The e.r.ps triggered by verbal stimuli have been related to several different aspects of language processing[1]. For example, the N400 component, peaking around 400 ms post-stimulus, appears to be a sensitive indicator of the semantic relationship between a word and the context in which it occurs. Words that complete sentences in a nonsensical fashion elicit much larger N400 waves than do semantically appropriate words or non-semantic irregularities in a text[2,3]. In the present study, e.r.ps were recorded in response to words that completed meaningful sentences. The amplitude of the N400 component of the e.r.p. was found to be an inverse function of the subject's expectancy for the terminal word as measured by its 'Cloze probability'. In addition, unexpected words that were semantically related to highly expected words elicited lower N400 amplitudes. These findings suggest N400 may reflect processes of semantic priming or activation.

Late negative e.r.ps resembling the N400 have been observed in experiments that required subjects to make decisions about words based on their semantic attributes,[4–7] and Stuss *et al.*[8] noted an N400-like component following isolated, single words or pictures that required naming. One likely interpretation of these findings would assume that N400 amplitude reflects the extent to which a word is unpredictable or unexpected, regardless of whether or not it is incongruous with a preceding context. Since word expectancy influences how rapidly and accurately words are accessed, recognized and understood[9], a physiological index of this process would have considerable usefulness for revealing the structure of language comprehension mechanisms.

We examined this relationship by recording e.r.ps to words that completed sentences in a meaningful way but varied systematically in the degree to which they were expected. The sentences were selected from a set in which the degree of expectancy for alternative terminal words had been determined using the 'Cloze' procedure; that is, by requiring a large group of subjects to fill in the missing terminal word[10]. A word's Cloze probability is defined as the proportion of subjects using that word to complete a particular sentence. The experimental design called for words having different Cloze probabilities (hi, med, or lo) to be placed at the ends of sentences having one of three levels of contextual constraint (hi, med, or lo). Highly constrained sentences were those that led to very predictable endings, while sentences of low constraint did not induce such strong expectations (for examples see Fig. 1*A*).

A total of 321 sentences were presented, one word at a time, on a video terminal controlled by a microcomputer. Words were presented once every 700 ms for a duration of 132 ms. Fourteen subjects were instructed to read the sentences silently in order to answer a questionnaire about their contents at the end of the experiment. Scalp electrical activity was recorded using non-polarizable electrodes from frontal (Fz), central (Cz), parietal (Pz), and occipital (Oz) midline locations and from symmetrical sites over the anterior temporal (AT) and posterior temporal (PT) regions of the left (L) and right (R) hemispheres, each referred to linked mastoids. Eye movements and blinks were monitored via infraorbital and external canthal electrodes. The midline recordings were amplified with a bandpass of 0–40 Hz; the lateral recordings and electrooculogram with a 0.01–40 Hz bandpass.

The e.r.p. waveforms in Fig. 1*B* show that highly probable words at the ends of highly constrained sentences were followed by a broad, late positivity (hi/hi, solid tracing). In contrast, the low probability words elicited a posteriorly distributed negative

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A

hi/hi	He mailed the letter without a stamp.
hi/lo	The bill was due at the end of the hour.
med/hi	She locked the valuables in the safe.
med/med	Too many men are out of jobs.
med/lo	The dog chased our cat up the ladder.
lo/hi	There was nothing wrong with the car.
lo/lo	He was soothed by the gentle wind.

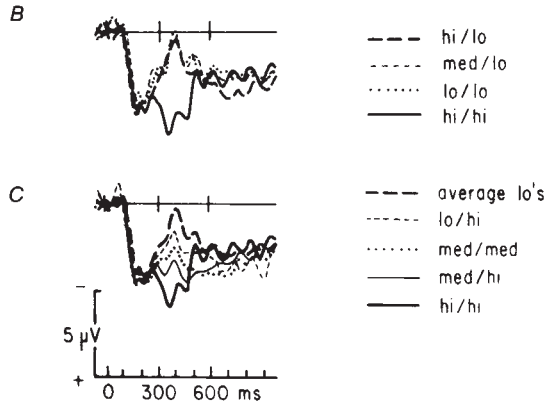

Fig. 1 *A*, An example from each of seven classes of sentences that varied in degree of contextual constraint and in the Cloze probability of the terminal word. There were 40–50 of each sentence type. On the left are shown the levels of contextual constraint/Cloze probability for each class. These two dimensions are not wholly independent, since only the more highly constrained sentences have the possibility of being terminated by words of very high Cloze probability. Thus, the level of Cloze probability considered to be 'hi' was relative to the level of contextual constraint, having average values of 0.92, 0.63 and 0.29 for the hi/hi, med/hi and lo/hi sentences, respectively. All of the low Cloze probability sentences, however, had average values of less than 0.03. *B*, Superimposition of grand average e.r.ps (across 14 subjects) from the Pz electrode to the low Cloze probability words terminating sentences of high, medium and low contextual constraint, together with the e.r.p. to high Cloze probability words completing highly constrained sentences. *C*, Grand average e.r.ps to terminal words of varying Cloze probability terminating sentences of high, medium and low contextual constraint. The e.r.p. to low Cloze probability words was averaged across the three levels of contextual constraint shown in *B*.

A

(best)	Don't touch the wet paint.
(unrelated)	Don't touch the wet dog.
(best)	He liked lemon and sugar in his tea.
(related)	He liked lemon and sugar in his coffee.

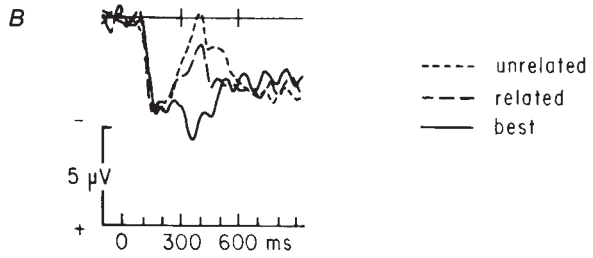

Fig. 2 *A*, Two examples of sentences with high contextual constraints completed by low Cloze probability words. Above each experimental sentence is the same sentence terminated by its 'best completion', which was or was not semantically related to the word that was actually presented in the sentence below. *B*, Grand average e.r.ps from Pz for the best completions (solid waveform), the semantically related (large dashed waveform) and the semantically unrelated (small dashed waveform) low Cloze probability words.

component (N400) that was superimposed upon the positive shift. The N400 amplitude to the low probability endings did not vary significantly over the three levels of contextual constraint (compare dotted and dashed tracings).

The N400 was measured as the mean amplitude over 300–500 ms post-stimulus, relative to a 50 ms prestimulus baseline. In general, the N400 amplitude was more sensitive to Cloze probability than to the degree of contextual constraint. For example, the e.r.ps to lo/hi versus med/med words, which were very similar in average Cloze probability (0.29 versus 0.23) but completed sentence fragments of low and medium constraint, respectively, did not differ significantly in N400 amplitude. On the other hand, comparisons of the e.r.ps to high, medium and low probability words terminating sentences of medium constraint revealed larger N400 amplitudes to the less probable words [main effect of ending Cloze probability $F(2,26) = 12.94$, $P < 0.001$; ending × electrode $F(14,182) = 6.07$, $P < 0.001$].

Inspection of the e.r.ps to all types of terminal words (Fig. 1*C*) revealed a gradient of potential, with the greatest positivity following hi/hi words and a progressively larger negativity (N400) elicited by words of decreasing Cloze probability. This relationship between Cloze probability and N400 amplitude was also evidenced by the product–moment correlations between the two measures across the seven classes of word endings [for the grand average e.r.ps, Fz, 0.80; Cz, 0.90; Pz, 0.92; Oz, 0.92; L.AT, −0.82; R.AT, 0.94; L.PT, 0.88; R.PT, 0.97]. At the parietal site, this correlation calculated from individual subjects' data averaged 0.70 (0.21) and reached significance in seven subjects (d.f. = 5, $P < 0.05$). This correlation also held when the e.r.p. data were collapsed across the different levels of contextual constraint and re-averaged according to the Cloze probability of the final words [Fz, 0.87; Cz, 0.91; Pz, 0.94; Oz, 0.92; L.AT, −0.60; R.AT, 0.75; L.PT, 0.91; R.PT, 0.93]. The negative correlation observed at the left anterior temporal site could result from this scalp region being located on the opposite side of the dipole field of the N400 generator.

The systematic decline in the N400 amplitude as a function of increasing Cloze probability indicates that semantic incongruity is not a necessary condition for N400 elicitation. Instead, N400 amplitude appears to vary systematically as an inverse function of word expectancy, operationally defined here in terms of Cloze probability.

The influence of context on word recognition has been attributed to the automatic priming or activation of semantic networks, as well as to slower, attention-directed processes[11–13]. Within such a framework, a sentence fragment primes (that is, activates for faster access) semantically related words whether or not they form acceptable sentence completions. In addition, sentence frames may result in the activation and retrieval of appropriate schemata[14–16]. If the N400 reflects some aspect of this semantic activation, its amplitude should vary according to whether or not an unexpected terminal words is semantically related to the most expected ending of that sentence (that is to the 'best completion' of the activated schema).

We tested this prediction by reanalysing the e.r.ps to low Cloze probability words that completed highly constrained sentences, now segregating them according to whether or not the terminal word was related to the best completion (BC) of the sentence in which it occurred. The degree of semantic relatedness had been determined by asking a different group of 25 subjects to rate each word pair on a 5-point scale. The mean ratings were 4.03 (0.70) and 1.95 (0.73) for the related and unrelated word pairs, respectively. Sample sentences are shown in Fig. 2*A*. N400 amplitude was indeed sensitive to the semantic relationship between the eliciting word and the expected best completion (Fig. 2*B*), with larger N400s following words that were unrelated to the BC [main effect of semantic relatedness

$F(1,13) = 17.37$, $P < 0.001$; relatedness × electrode $F(7,97) = 3.14$, $P < 0.001$].

These results are in agreement with the hypothesis that the N400 component reflects the extent to which a word is semantically primed, rather than its being a specific response to contextual violations. If the N400 amplitude proves to be a valid index of semantic priming, it should become possible to investigate the timing and spread of activation within semantic networks and knowledge schemata and to identify automatic and attentional components of processing.

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Cell determination and regulation during development of neuroblasts and neurones in grasshopper embryo

Paul H. Taghert, Chris Q. Doe & Corey S. Goodman

Department of Biological Sciences, Stanford University, Stanford, California 94305, USA

The embryonic development of the central nervous system (CNS) involves the generation of an enormous diversity of cellular types arranged and interconnected in a remarkably precise pattern. In each hemisegment of the grasshopper embryo, the ectoderm generates a stereotyped pattern of 30 neuronal precursor cells, called neuroblasts (Fig. 1)[1]. Each of these stem cells makes a stereotyped contribution of 6–100 progeny to the ~1,000 different neurones, each cell identifiable according to its unique morphology, physiology and biochemistry. What are the contributions of cell interactions and cell lineage to the generation of this diversity and specificity of identified neurones in the grasshopper CNS? Here we report on cell ablations with a laser microbeam at different stages of development. Our results suggest the importance of cell–cell interactions in the determination of ectodermal cells to become identified neuroblasts[2]. However, once a neuroblast begins to divide, then cell lineage appears to play an important role in the determination of its stereotyped family of neuronal progeny[3]. Furthermore, cell-specific interactions continue to play an important role as neurones, according to their mitotic ancestry, recognize and interact with other differentiating neurones in their environment[4–6].

Within the neuroepithelium in each segment of the grasshopper embryo, there arise 61 neuronal precursor cells, called neuroblasts (NBs), arranged in two symmetric plates of 30 NBs each and one median neuroblast (MNB) (Fig. 1)[1]. We can identify each NB by its position within the neuroepithelium; moreover, many NBs can now be uniquely identified by the highly stereotyped family of identified neurones they produce[3,7–10]. Each NB is a stem cell which divides repeatedly to generate a chain of ganglion mother cells. Each ganglion mother cell then divides once more to generate two ganglion cells in a chain of cell doublets that then differentiate into neurones. The 30 NBs in each hemisegment largely generate the ~1,000 neurones in each thoracic hemiganglion and the ~250 neurones in each abdominal hemiganglion; most if not all of these neurones can be uniquely identified according to their characteristic morphology, physiology and biochemistry. The number of neuronal progeny appears to be specific for individual NBs; for example, NB 7–3 produces 6 progeny[10] whereas the MNB produces ~100 progeny[6–8]. Furthermore, the identities of the progeny appear to be specific for individual NBs; the fate of individual neurones is highly correlated with their specific positions in the family trees of particular NBs[6–10].

The progeny of NBs form coherent families of neurones that are inserted into the developing CNS in characteristic positions. The determination of an individual neurone might be due to its position in the NB family tree, or alternatively to its position in the developing ganglion and thus its reproducible interactions with its neighbours. Selective ablation of identified cells with a laser microbeam can help to distinguish between these alternatives. For example, NB 7–3 gives rise to six progeny (Fig. 1). In the abdominal segments (A4–A6), the first two NB 7–3 progeny are called S1 and S2; following their differentiation they can be specifically stained with an anti-serotonin antibody (Fig. 2). They are the only prominent serotonin-immunoreactive neurones in these segments. Furthermore, S1 and S2 can also be individually identified by their distinctive morphologies after intracellular dye injections (Fig. 1)[1]. We ablated NB 7–3 *in ovo* just before it begins its first cell division to test if some other NB could produce neurones with either the biochemical or morphological phenotypes of S1 and S2 (Fig. 2). We also ablated other NBs at the same stage to test if interactions with neighbouring NBs or their progeny were necessary for NB 7–3 to produce neurones S1 and S2.

NBs were identified in a 30%[11] embryo through the dechorionated eggcase under brightfield optics, and ablated with a pumped-dye laser (Fig. 2). Experimental and control embryos were examined at three different stages with three different techniques: at 33% with Nomarski optics to look for NB 7–3; at 50% with intracellular dye injections of Lucifer Yellow to look for cells with the distinctive morphology of neurones S1 and S2; and at 70% with the anti-serotonin antibody to look for cells with the distinctive serotonin-immunoreactivity of neurones S1 and S2.

When NB 7–3 was ablated before it began its first cell division and the embryos were assayed at 33% (the following day) for the presence or absence of the NB, we found that in approximately 80% of the cases ($n > 20$) no regulation took place. When regulation does occur, a new NB 7–3 is present as are all neighbouring NBs. When embryos were assayed at 70%, in 7 of 10 cases no serotonin-immunoreactive neurones were present on the experimental side of the ganglion (Fig. 2). In these embryos, the homologous S1 and S2 neurones from the contralateral NB 7–3 were always present and had the normal morphological and biochemical features. These observations suggest that when a NB is ablated before it begins its first cell division, regulation does not usually take place. In addition, progeny of neighbouring NBs do not differentiate with either the morphological or biochemical properties of neurones S1 or S2. The partial (20–30%) regulation we observe following NB ablation is likely to result from the recruitment of a neighbouring ectodermal cell, as described in the *in vitro* experiments below. In three other cases in which NB 7–3 was ablated, exhaustive intracellular dye injections at 50% of every other cell body in the region where S1 and S2 normally appear revealed no neurones with the distinctive morphology of S1 and S2 on the experimental side; S1 and S2 were easily found on the control side, and did

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