Presynaptic mechanism for long-term potentiation in the hippocampus

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Experiments analysing the statistical properties of synaptic transmission, before and after the induction of long-term potentiation (LTP), suggest that expression of LTP largely arises in a presynaptic mechanism—an increased probability of transmitter release.

**Long-term** potentiation (LTP) is a long-lasting use-dependent increase in the efficacy of excitatory synaptic transmission in the brain that is thought to underlie certain forms of learning and memory. Although the trigger for LTP is generally agreed to occur in the postsynaptic cell, the site at which it is expressed is still disputed. Some authors have suggested that the mechanism of LTP is enhanced neurotransmitter release, others that it is increased postsynaptic sensitivity to transmitter, and still others that both may occur, perhaps in sequence. Here we describe experiments analysing the statistical properties of synaptic transmission, before and after the induction of LTP, in both hippocampal cultures and patch-clamped slices.

**Quantal analysis**

Since the classic work of Del Castillo and Katz, the standard way to determine whether a phenomenon is pre- or postsynaptic has been to carry out a quantal analysis. Such an analysis provides estimates of the probability of release, of the quantum of neurotransmitter, and of the probability of release. Synaptic transmission becomes more efficacious if a higher proportion of the release sites is activated. In standard treatments, an increase in either the number of release sites or in the probability of release is observed. These changes in the quantal properties of the synapse are collectively referred to as postsynaptic potentiation.

Quantal analysis has, however, proved difficult in the central nervous system for three main reasons. First, only rarely can one find a pair of cells that are monosynaptically connected. The formalism for quantal analysis can be extended to include some situations in which more than one input to a neuron is stimulated, but such an extension is uncertain for the present case. Second, the quantal properties of the postsynaptic cell cannot be measured directly. Third, the quantal properties of the presynaptic cell cannot be measured directly.

**Analysis in dissociated neurons**

In a preliminary series of experiments in culture, we used the nystatin perforated patch technique to observe m.e.p.s.c.s in a voltage-clamped neuron before and after exposure to a bath solution containing no Mg. Such perfusion causes cells in the dish to fire spontaneous bursts of action potentials repeatedly and should have potentiated many synapses. Assuming LTP had been induced in a significant number of the neuron's synapses, we should have observed an increase in the mean size of m.e.p.s.c.s if expression of LTP is postsynaptic. No detectable change in m.e.p.s.c. size was found up to 90 min after the induction of massive activity in the culture, suggesting that expression of LTP is not postsynaptic. The difficulty with this experiment was, of course, that we could not be sure that the synapses whose m.e.p.s.c.s we recorded had undergone LTP. Therefore, we turned to a more conventional protocol.

At the outset we failed to observe LTP in 42 neurons when whole cell recording methods were used, and therefore suspected that some critical cytoplasmic factor was being washed out of the cells. Accordingly, all our later experiments used the nystatin perforated patch technique to record from both pre- and postsynaptic neurons.

Figure 1 shows an experiment in which an action potential was repetitively fired (at 0.1 Hz) in one neuron, and the resulting synaptic current was recorded under voltage clamp in a neighboring neuron (panel a). Panel b plots the amplitude of the current as a function of time during the experiment. For the first series of stimuli (before the arrow) the mean peak conductance was 1.62 nS, the standard deviation was 0.14 nS and on 97 occasions the presynaptic action potential failed to evoke a postsynaptic response. At the arrow the bathing medium containing 10 mM Mg was (to prevent any possible potentiation during the test stimulation) was exchanged for one with added Mg, the postsynaptic neuron was switched from voltage clamp to current clamp, and the presynaptic neuron was stimulated three times for 2 s at 20 Hz. After this stimulation, the bathing solution (with 10 mM Mg) was reintroduced, and the presynaptic voltage clamp was re-established, and the 0.1 Hz stimulation of the presynaptic cell resumed. Following the tetanic stimulation, the mean synaptic current amplitude was 3.43 nS, the standard deviation 0.97 nS, and no failures in transmission occurred in 128 trials. The potentiation factor f in this case was (3.43/1.62) = 2.1. The amplitude histograms for pre- and posttetanus synaptic current are shown in Fig. 2.

The following results argue in favour of the phenomenon in Fig. 1 being LTP. (1) The potentiation was long-lasting. For example, the e.p.s.c. in Fig. 1 was still at the same, elevated level 60 min after the tetanus. In two other cells the roughly threefold potentiation lasted for 80 and 90 min, respectively (which point the cells were lost). (2) Potentiation could only be induced in a low Mg concentration, as expected from the triggering characteristics of LTP (3). (3) Potentiation was not an...
FIG. 1 Long-term potentiation of excitatory synaptic transmission between a pair of rat hippocampal neurones in dissociated cell culture. a, Superimposed action potentials recorded in the presynaptic cell (upper traces) and corresponding e.p.s.c.s recorded simultaneously in the postsynaptic cell (lower traces). Left-hand panels were recorded shortly before, right-hand panels about 10 min after a series of three 3-s tetani at 20 Hz separated by 30 s, performed with the postsynaptic cell current clamp. All pre- and post-tetanus recordings were made with 10 mM Mg²⁺ solution (see below) in the bath; the tetani were delivered after briefly changing to a bath solution lacking Mg²⁺. Action potentials were fired by injecting into the presynaptic cell a 10-ns 1-nA current step at 1 Hz. The presynaptic cell's resting membrane potential was near −60 mV; the postsynaptic cell was voltage clamped at −60 mV. Arrows in the right-hand panels indicate spontaneous polysynaptic activity that was recorded simultaneously in both cells (the current trace, lower panel, goes off-scale). Such activity was much more frequent after tetanus. Calibration bars refer to both right- and left-hand panels. The dotted lines in the upper panels indicate 0 mV.

b Plot of peak e.p.s.c. amplitude as a function of time for the same experiment shown in a. The arrow indicates the time at which the sequence of tetani was applied. Immediately before this the bath was changed to a 0 mM Mg²⁺ solution, and then immediately afterward back to the control 10 mM Mg²⁺ solution. The straight line through the data points represents the mean amplitudes over the time window indicated by the horizontal positions of the lines.

METHODS. Pyramidal neurones from lobus CA1 and CA3 of the hippocampus of newborn Long Evans rat pups were maintained in dissociated cell culture in previously described media, except that 50 µM 6-2-amino-5-phosphonovaleric acid was added to the culture medium after about a week to block possible potentiation due to spontaneous firing. For electrophysiology, the bath solution comprised (in mM) NaCl (137), KCl (4.2), CaCl₂ (3), MgCl₂ (10 or 0), d-glucose (10), HEPES-NaOH buffer (5), pH 7.3, osmolality adjusted to 310 mOsm with sorbitol, to which was added 1 µM glycine, 1 µM strychnine and 100 µM bicuculline. The tips of the patch electrodes were filled with a solution comprising (in mM) KCl (150), (3), EGTA (10), HEPES-KOH buffer (10), pH 7.2, and electrodes were also filled with the same solution plus 100 µg ml⁻¹ nystatin, diluted from a stock of 20 mg nystatin per ml of DMSO. Patch electrodes were Silgared and polished and had resistances of 1-3 MΩ with these solutions. After obtaining GD seals on a pair of cells, test pulses were applied until the access resistances stabilized (typically 30-50 min after forming the seals); access was checked for constancy throughout the experiment. Recordings were made with an Axopatch 1A, filter setting 1 kHz. The bath was perfused at about 1 ml min⁻¹ (bath volume 1 ml). Electrode offset potentials were within 2 mV at the end of the experiment. All measurements were made at room temperature (23-25 °C).

FIG. 2 Frequency histograms of e.p.s.c. conductance before and after inducing LTP are best fitted by assuming that LTP expression originates in an increase in release probability, p. a, Control (pre-tetanus) histograms. b, Post-tetanus histograms. (Same experiment as in Fig. 1.) In both a and b the right panel is the normalized cumulative histogram of the histogram in the left panel. In a the filled circles represent a binomial distribution fitted as described in the text. In b the filled circles were calculated from the distribution in a assuming that only p is scaled by the potentiation factor f, with no free parameters. The dotted line and the broken line were calculated assuming that the parameters N and a, respectively, are scaled by f.
 artefact due to changing recording conditions. For example, the
presynaptic action potential remained constant (Fig. 1a), and
the bath was perfused at a continuous rate, preventing changes
in the concentrations of possible modulatory substances.26

Long-lasting potentiation was seen in 4 cell pairs out of 13
stable, connected pairs studied with the perforated patch tech-
nique. However, even in experiments in which the evoked e.p.s.c.
was not potentiated, the above protocol usually caused a long-
lasting increase in the general polysynaptic activity of cells in
the culture. An example of this is visible in Fig. 1a, after tetanus
(arrows). This is probably due to the potentiation of synapses
other than those between the cell pair under study, and may
arise in heterogeneity in the ability of synapses to support LTP27.

Our strategy was to carry out a quantal analysis of the pre-
tetanus responses then to test, without estimating additional
parameters, three alternative mechanisms for the potentiation
by predicting the amplitude histogram obtained after potenti-
ation had developed. The potentiated response in Fig. 1 could
be due to \( f = 2.1 \) fold scaling of \( N \) (the number of release sites),
\( p \) (the quantal release probability) or \( a \) (the mean quantal size);
as each of these factors enters the equation for the synaptic
current size histograms in a different way, scaling each makes
different predictions about the shape of the histograms. Thus
we may determine which of the alternative theories best accounts
for the data.

The probability of observing a synaptic current of a particu-
lar amplitude describing the amplitude histogram is given by
the binomial distribution\(^{25} \) suitably modified to take account of the
observed variability in the m.e.p.s.c. size\(^{26} \). If \( a \) is taken to be
the average value for the quantal size in culture (0.8 nS), the
pre-tetanus binomial parameters \( N \) and \( p \) can be estimated from
the standard equations for the mean \( m = 1.62 \) nS and variance
\( \sigma = 1.073 \) nS\(^2 \) of the amplitude histogram (Fig. 2a):

\[
\begin{align*}
m &= aNp \\
v &= a^2Np(1-p) + a^2Npc_m^2,
\end{align*}
\]

The second term gives the contribution to the overall vari-
ability of fluctuations in the m.e.p.s.c. size; the coefficient of vari-
ability of m.e.p.s.cs is \( c_m \) and has an average value of 0.5 in culture
and 0.42 in slices. Solving these equations yields \( N = 5 \) and
\( p = 0.42 \). As \( N \) should be an integer, \( N \) was constrained to
5 (rather than the calculated 4.8) and \( p \) was set to 0.37 (instead
of 0.42).

The predicted and observed pre-tetanus histograms are shown
superimposed in Fig. 2a; they are not significantly different at
the 0.2 level (\( x^2 \) goodness-of-fit test); that is, worse fits would
occur by chance more than one time in five even if the histograms
were generated by the same underlying mechanism. Although
we could not in these experiments collect sufficient quantities of
pre-tetanus data to carry out a very accurate quantal analysis,
the fit exhibited in Fig. 2a confirms our previous conclusion
that the Del Castillo and Katz formalism, with the m.e.p.s.c.
variability included, accounts for the observed amplitude
histogram.

Figure 2b shows the result of assuming that the mechanism
of LTP is an \( f \)-fold increase in the release probability \( p \),
\( N \) and \( a \) unchanged; the predicted histogram that results
is superimposed on the observed post-tetanus amplitude histo-
gram. The predicted and observed histograms are not significa-
cantly different at \( P < 0.2 \). The two other alternative mechanisms
an \( f \)-fold increase in \( N \) or in \( a \) make predictions that differ
from the observed histograms at \( P < 0.05 \) and 0.01 levels of
significance, respectively (dotted and broken lines in Fig. 2a).

A convenient way to compare predicted and observed histo-
grams uses the coefficient of variation (and its squared value)
\( c = \sqrt{\frac{\sigma}{m}} = \sqrt{\frac{\sigma}{m}} \) introduced in this context by Del Castillo and Katz,\(^{25} \) and
modified here to take account of m.e.p.s.c. variability:

\[
h(N, p) = (1 + \frac{c_m^2}{m} - p)/(Np).
\]

Note that \( a \) cancels in the right-hand side of this equation.

\[\text{FIG. 3. Long-term potentiation of excitatory synap-
 tic transmission in a patch-clamped hippocampal slice. a, Superimposed}
\text{e.p.s.c.s, elicited by stimulation of the Schaffer collateral pathway at 0.5 Hz.}
\text{recorded from a cell in area CA1 before (left) and after (right) tetanus-induced LTP.}
\text{The neuron was whole-cell voltage-clamped at the soma at -70 mV. Calibration bars refer to both paradigms. b, Plot of the amplitudes of evoked e.p.s.c.s as a}
\text{function of time during the experiment. At each arrow the patch amplifier was switched to current clamp mode and two 200-ms 100 Hz tetani were}
\text{delivered 5 s apart. The amplifier was then returned to voltage clamp mode and testing resumed. The straight lines through the data points rep-
 resent the mean amplitudes over the time windows indicated by the horizontal positions of the lines.}
\text{METHODS. Transverse slices (300-400 \mu m) were cut from the hippocampal}
\text{slice of 14-21-day-old Long Evans rats using a vibrotome; submerged and}
\text{continuously perfused at 1-2 ml min}^{-1} \text{ with oxygenated Krebs solution comprising (in mM) NaCl (120), KCl (3), MgCl}_2 (1.2), CaCl}_2 (2.5), \text{NaHCO}_3 (23), \text{NaH}_{2}PO}_4 (1.2), \text{D-glucose (11), to which} 100 \mu M\text{ bicuculline was added. Patch electrodes}
\text{containing (in mM) Ca-methylene-pyrophosphate (120), CaCl}_2 (5), \text{EGTA (0.5), MgCl}_2 (2), Mg-ATP (2), cobalt (10), HEPES-CaSO}_4 buffer (10), pH 7.3. Gigahm sealing}
\text{seals were obtained with unpolished electrodes (resistances 3-5 M\Omega) on the}
\text{soma of cells in region CA1 and recordings made in the whole-cell with an Axopatch 3A, filter setting 2 Hz. A monopolar stimulating}
\text{electrode (35 \mu m diameter, teflon-coated Pt-Ir wire) was positioned 200-300 \mu m away from stratum radiatum and the stimulus intensity set just above the level that}
\text{gave mostly failures of synaptic transmission; this setting was subsequently}
\text{left unchanged throughout the experiment. The stimulus duration for}
\text{the frequency stimulation was 100 \mu s for tetani it was 200 \mu s.}
\text{Electrode potentials were within 2 mV at the end of the experiment. Experiments were performed at room temperature (23-25°C).} \]
below, that takes account of the possible nonuniformity of the stimulated inputs to a neuron. Second, we have simply applied the standard equations of quantal analysis, and have relied upon goodness-of-fit tests to determine if the application of these equations is valid. These approaches are described in turn.

The first approach is best described by presenting a graph that plots a quantity $r$ as a function of the factor $f$ by which the mean e.p.s.c. size is potentiated. The quantity $r$, like $f$, is determined directly from the experimental data and is defined as the ratio

$$r = h / h_0$$

where $h$ (defined earlier in equation (1)) is the squared value of the coefficient of variation before a tetanus and $h_0$ is the corresponding quantity in the potentiated slice. In the limiting case where $p$ is small (the Poisson limit), $r$ is, according to equation (1), equal to $f$ if the mechanism of LTP is an $f$-fold change in either $N$ or $p$ (synaptic mechanism) and equal to 1 if LTP results from a scaling of $a$ (postsynaptic mechanism). Figure 4 shows such an $r/f$ plot for 10 slice experiments and unambiguously accords with the predictions of the postsynaptic mechanism. Note that in two cases $f$ was less than unity; in these experiments the tetanus was applied with 100 nM D-APV in the bath, which resulted in a long-lasting depression.

The $r/f$ plot can be generalized to many situations with multiple inputs to a neuron in which a standard quantal analysis is not applicable. It can be shown in these cases that points will fall on or to the left of the diagonal ($r = f$) if the LTP mechanism is presynaptic (an increase in either $N$ or $p$), and will fall on a horizontal line ($r = 1$), or between that line and the diagonal, if the mechanism is postsynaptic (an increase in $a$). However, analysis reveals a special case for which the $r/f$ plot does not correctly distinguish between a pre- and postsynaptic mechanism for LTP. Suppose that individual postsynaptic membranes could exist in only two states, ‘on’ and ‘off’; for an ‘off’ synapse release of a quantum of neurotransmitter would result in essentially no response, and for an ‘on’ synapse, each quantum would give a standard-sized response. The mechanism of LTP in this model would be the postsynaptic switching (for example, by increasing the sensitivity of receptors in the postsynaptic membrane) from ‘off’ to ‘on’. Such a switch would appear in our analysis to be an increase in $N$, and thus to be presynaptic, whereas in fact the site of LTP expression would be postsynaptic. From our slice experiments alone we would not be able to distinguish between the presynaptic mechanism and this particular postsynaptic mechanism. The observations in culture, however, are not consistent with the postsynaptic mechanism because LTP there resulted from an increase in the release probability $p$, not $N$.

We conclude that our observations in slices, like those in culture, are consistent with the expression of LTP being primarily postsynaptic. Because points fall along the diagonal, indicating that release is described by the Poisson limit, we cannot here, as we could in some of the experiments on neuron pairs in culture, distinguish between effects on $N$ and $p$.

An alternative way to test our conclusion is to carry out a full quantal analysis of transmission in these slice experiments: if the mechanism is indeed postsynaptic and the quantal parameters sufficiently constant to give data points that fall along the diagonal in the $r/f$ plots, then the distribution of quantal current sizes should be well described by the standard equations of quantal analysis, and one should be able to predict the distribution of potentiated synaptic current sizes with the quantal parameters obtained from the unpotentiated distribution. If the conclusion is incorrect, then the observed distribution of synaptic current sizes should be a sum of Poisson distributions, and may not, in general, be adequately described by a single Poisson distribution.

Figure 5a shows a slice in which the postsynaptic response for a small stimulus is plotted as a function of time

**Analysis in hippocampal slices**

We have taken two approaches to extending our analysis to the hippocampal slice. First, we have used a procedure, described
during the experiment. Note that charge, rather than amplitude, is measured because the former is a quantity less sensitive to cable attenuation in the dendrites. At the point indicated by the first arrow, the inputs to the cell were stimulated repetitively but the postsynaptic neuron was maintained by the voltage clamp at –70 mV; the response did not potentiate after this procedure, as expected for LTP. At the second arrow, the cell's inputs were again stimulated while the cell was held at –20 mV, a value that should maximize calcium influx through the NMDA receptor channels and produce LTP. The mean response after this tetanus was potentiated by a factor f = 2.84. This particular experiment was chosen for illustration of the full quantal analysis because failures were apparent in the control records, which provides an additional check on the validity of the fitted parameters.

Figure 5b shows the histogram of observed synaptic responses for this experiment with superimposed predictions of the Poisson distribution, using appropriate values for a and Np estimated from the mean and variance of the raw data, and again taking into account the measured variance in m.e.p.s.c. size. A χ² goodness-of-fit test detects no difference between these distributions at the 0.2 significance level. The quantal size estimated by the ratio variance/mean for this experiment is 2.1 ± 0.37 pC mV⁻¹ before (and after) the tetanus. For six experiments, the average quantal size was 1.35 ± 0.37 pC mV⁻¹ before the tetanus and 1.41 ± 0.28 pC mV⁻¹ in the potentiated slice (mean ± s.d.). These estimates should be compared with the mean value of a, directly determined in slices of 1.5 ± 0.4 pC mV⁻¹ with a standard deviation of 0.5 ± 0.4 pC mV⁻¹ (ref. 20). The value for a estimated from the histogram is thus within the range of quantal sizes determined independently.

If the pre-tetanus value for Np is multiplied by f, the predicted histogram, superimposed on the experimental one after potentiation, is shown in Fig. 5c. Again the χ² goodness-of-fit test detects no difference between the observed and predicted histograms at the 0.2 significance level. If a, rather than Np, is multiplied by f, the predicted and observed histograms differ at the 0.01 significance level, so the differences are statistically significant (Fig. 5c; smooth curve). Similar results were found in other five experiments in which we carried out full quantal analyses. We conclude that the potentiation mechanism is presynaptic, and that either N or p increases following the tetanus.

The longest LTP observed in our slice experiments is 98 min, and our conclusions hold up to that time. We cannot, however, exclude the possibility that slower developing phases of LTP use some different mechanism. Because our experiments were done at room temperature (23–25°C), and we do not know the temperature coefficients of the various possible phases of LTP, we cannot even place limits of the validity of our conclusions for the hippocampus at body temperature. From just after the tetanus up to 1 h after, only a single phase of LTP was apparent in our experiments, and the statistical properties of the postsynaptic response were essentially invariant.

Discussion

Our conclusion is that the mechanism of LTP is presynaptic rather than postsynaptic, and probably involves N instead of p. We have not, however, attempted to place specific limits on the extent to which intermediate cases, in which two or three of the quantal parameters are affected by LTP, might hold. Specifically, we cannot exclude the possibility that some small fraction of LTP is postsynaptic, or that both N and p increase with the effect predominating. Because the potentiation factor f enters squared if the LTP mechanism is postsynaptic and linearly if it is presynaptic, a large contribution from the postsynaptic site can be excluded. N as well as p could be affected significantly, and we would not be able to detect this.

Our conclusion that the expression of LTP is, under conditions of our experiment, mainly presynaptic, directed our attention to mechanisms whereby retrograde signals from the postsynaptic cell cause enhanced presynaptic release of neurotransmitter.

Note added in proof: Our observations and conclusions are in accord with those reported by R. Mainown and R. W. Tsien. 

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Detection of diffuse interstellar bands in the infrared

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The diffuse interstellar bands (DIBs) are absorption features caused by interstellar matter but not identified with any known atomic or molecular lines. Since 1975, when 39 such bands were known, many more have been detected, and in 1988, about 86 were listed. They range in frequency from 4.430 Å to 15.9 Å, and in the infrared wavelength range 6.850 Å (ref. 1), the strong 15.9 Å and 13.2 Å bands are also detected. These bands show a variety of behaviors, including: (1) incomplete removal of telluric lines; (2) intrinsic stellar features that are not strictly compensated (for example, Fig. 14 shows He I features visible in the ratio of the spectra of object 6 and a reference of spectral type B1 III). Both of these kinds of features can be identified because they are prominent in the raw spectrum of DIBs and references; (3) interstellar features. We consider a feature to be interstellar if it is present in the most reddened stars (aplying a 3σ detection criterion) and absent in references, if its strength varies with E(B−V), and if its position from one line of sight to another can be explained by a reasonable Doppler shift. Typical interstellar clouds have a velocity dispersion of <50 km s⁻¹, which gives a maximum variation from one line of sight to another of 2 Å. We found two such features. The stronger band lies between 13,174 Å (7.582 cm⁻¹) and 13,176 Å (7.584 cm⁻¹) (wave-lengths λ are measured in air at standard temperature and pressure and wave-numbers ν are measured in vacuum: ν(Å) = ν(cm⁻¹) × 10⁶/n where n is the index of refraction of air, 1.000273) and has a full width at half maximum (FWHM) of 4.0 ± 0.5 Å. The wavelength range of the weaker band is 11,797 Å ± 200 Å. This led us to look for interstellar features in the near infrared.

Our observations were carried out on the 3.6-m Canada-France-Hawaii telescope at Mauna Kea using a Fourier-transform infrared spectrometer with a resolution of ~10⁶ (resolution element 0.9 cm⁻¹). From Herbig's, we selected reddened stars exhibiting DIBs in the visible. The stars studied are listed in Table 1—they have different spectral types as well as various values of colour excess, E(B−V). During the first run (September 1988), we studied two objects using the J, H and K filters and detected two possible interstellar features in the J filter but none in the others. In the second run (November 1989), we observed four objects using only the J filter.

To remove most of the telluric absorptions (mainly H₂O absorption bands), we divided the spectrum of an object by the spectrum of a reference star. A good reference should be located at the same air mass as the object and also be of the same spectral type. Moreover, to allow for correct removal of stellar features without cancelling truly interstellar ones, the standard star should show no reddening. We tried to fulfill these criteria as much as possible. The remaining features in the ratio spectra are of three kinds: (1) incompletely removed telluric lines; (2) intrinsic stellar features that are not strictly compensated (for example, Fig. 14 shows He I features visible in the ratio of the spectra of object 6 and a reference of spectral type B1 III). Both of these kinds of features can be identified because they are prominent in the raw spectrum of DIBs and references; (3) interstellar features. We consider a feature to be interstellar if it is present in the most reddened stars (applying a 3σ detection criterion) and absent in references, if its strength varies with E(B−V), and if its position from one line of sight to another can be explained by a reasonable Doppler shift. Typical interstellar clouds have a velocity dispersion of <50 km s⁻¹, which gives a maximum variation from one line of sight to another of 2 Å. We found two such features. The stronger band lies between 13,174 Å (7.582 cm⁻¹) and 13,176 Å (7.584 cm⁻¹) (wave-lengths λ are measured in air at standard temperature and pressure and wave-numbers ν are measured in vacuum: ν(Å) = ν(cm⁻¹) × 10⁶/n where n is the index of refraction of air, 1.000273) and has a full width at half maximum (FWHM) of 4.0 ± 0.5 Å. The wavelength range of the weaker band is 11,797 Å ± 200 Å. This led us to look for interstellar features in the near infrared.

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TABLE 1 Stars observed

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