Induction of synchronous oscillatory activity in the rat lateral amygdala in vitro is dependent on gap junction activity

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Abstract
Synchronized and rhythmic activity within the amygdala is thought to play a pivotal role in the generation of fear- and anxiety-related behaviour. The aim here was to determine the validity of the in vitro amygdala slice preparation to investigate the generation of rhythmic activity similar to that observed in vivo. Extracellular population activity recorded from the lateral nucleus of the amygdala in vitro showed significant enhancement of activity within the theta-band frequency (3–9 Hz) in the presence of kainic acid (100 nM; n = 18). Alterations in the patterns of oscillatory activity within the gamma frequency band (20–40 Hz) were observed in the presence of (RS)-3,5-dihydroxyphenylglycine (10 μM; n = 7) or carbachol (50 μM; n = 5). Theta frequency oscillatory activity was blocked in the presence of the gap junction blocker carbenoxolone (100 mM), whereas gamma frequency oscillatory activity showed increased variability in the dominant frequency of rhythmic activity. The results suggest that the neuronal circuitry of the amygdala in vitro is capable of generating and sustaining rhythmic activity and that intercellular communication via gap junctions may play a role in the synchronization of population activity underlying this oscillatory activity.

Introduction
Rhythmic oscillatory activity has been described throughout the central and peripheral nervous system. Oscillations in both areas have been shown to exhibit state dependence and are thought to underlie the integration and ‘binding’ of information (e.g. Singer, 1993; Varela et al., 2001) and generation of appropriate behavioural responses.

Oscillatory activity in vitro has been well documented in neocortical areas involved in perception and learning, such as the hippocampus (see Buszaki, 2002). The frequencies of population activity observed are thought to be dependent on the coherence of activity within local GABAergic circuits (Traub et al., 2004) and can be modulated by pharmacological alteration of intrinsic network activity. Within the hippocampus, gamma frequency-band oscillations are readily induced by application of cholinergic agonists (Williams & Kauer, 1997; Fisahn et al., 1998; Páhlalmi et al., 2004) and metabotropic glutamate receptor agonists (Whittington et al., 1995; Páhlalmi et al., 2004), and a recent study has shown that theta frequency activity can be induced within the diagonal band of the medial septum by application of kainic acid (KA; Garner et al., 2005). Furthermore, coherence of the oscillatory activity within GABAergic networks is thought to be mediated by the intercellular communication afforded by gap junctions (Tamas et al., 2000; Hormuzdi et al., 2001), electrotonic transmission via gap junctions leading to a predisposition for synchronized firing between coupled cells.

Within the amygdala, oscillatory activity in the theta-band frequency has been demonstrated in vivo during formation of emotionally linked conditioned behavioural responses (Paré & Collins, 2000). Synchronized activity within the lateral amygdala (LA) is thought to underlie integration of sensorimotor input and formation of appropriate outflow to the central nuclei (e.g. Pelletier & Paré, 2004), which projects to the major centres underlying the production of behaviour along (see Samson et al., 2005).

Data presented here describes the generation of oscillatory activity within the lateral nucleus of the amygdala in vitro through pharmacological activation of kainate and metabotropic glutamate receptors and, furthermore, that the generation of this synchronous activity may be dependent on intercellular communication via gap junctions.

Materials and methods
Coronal amygdala slices, 400 μm thick, were prepared from male Wistar rats (150–200 g) in accordance with the UK Animals (Scientific Procedures) Act 1986. Animals were terminally anaesthetized with halothane and decapitated. The brains were rapidly excised and sectioned in chilled (< 4 °C) artificial cerebrospinal fluid (aCSF), and coronal slices were cut using a vibratome. Slices were held at room temperature (22–24 °C) for 2 h prior to recording, then transferred and maintained in a constantly perfused and humidified interface chamber held at either 30 ± 1 °C or room temperature (22–24 °C). aCSF contained (in mM): NaCl, 124; KCl, 3; NaHCO₃, 26; NaH₂PO₄, 1.25; CaCl₂, 2; MgSO₄, 1; and d-glucose, 10; equilibrated to pH 7.4 with 95% O₂ and 5% CO₂.

Glass microelectrodes containing aCSF (resistance 2–6 MΩ) were visually positioned in the lateral nucleus of the amygdala and allowed to equilibrate prior to recording. Extracellular population activity was recorded and amplified using an Axoprobe 1A amplifier (Axon Instruments, USA) and Neurolog NL106 AC/DC amplifier; mains interference was removed with a 50 Hz noise eliminator (HumBug;...
Digitimer Ltd, UK). Data were digitized and acquired online using the PClamp software suite (Axon Instruments); sampling rate was 1–5 KHz.

Data were analysed using Spike2 software (CED, UK). To assess the rhythmic components of population activity, power spectra were constructed for 300 s epochs of data using the Spike 2 fast Fourier transform algorithm. Data were windowed using a Hanning taper and the frequency resolution was 0.12 Hz. Area power was calculated and is presented as mean ± SEM, bandwidth as modal frequency ± frequency at 2 SD of power. Statistical significance was determined using paired Student’s t-test and ANOVA; \( P < 0.05 \) was considered significant.

Drugs were prepared from frozen aliquots of stock solutions (×1000), diluted directly in aCSF to obtain the appropriate concentrations, and applied by perfusion. The kainate receptor agonist KA and the metabotropic glutamate receptor agonist (RS)-3,5-dihydroxyphenylglycine (DHPG) were obtained from Tocris (Bristol, UK); the cholinergic agonist carbamoylcholine chloride (carbachol), the gap junction blocker carbenoxolone (CBX) and the mineralocorticoid antagonist spironolactone were obtained from Sigma–Aldrich (UK).

Results

Perfusion with 100 nm KA induced rhythmic oscillations in the LA within the theta frequency range (3–9 Hz) in 13 of 18 slices tested. The onset of oscillatory activity was evident between 20 and 40 min from the start of KA perfusion. There was no significant change in

Fig. 1. KA induced theta frequency-band oscillations in the LA. (Ai) Focal wave recording taken at a slow time scale, showing increase in population activity induced by perfusion with 100 nm KA. (Aii) Graph showing average total power in the 3–9 Hz band (\( n = 18 \)); *\( P < 0.05 \). (Bi) Superimposed unfiltered traces showing increase in theta frequency activity induced by perfusion with 100 nm KA. (Bii) Spectral composition of activity in the LA during control and KA perfusion, performed on 300 s epochs of raw data (from the same experiment as shown in Bi). (Ci) Focal wave recordings (filtered 0–10 Hz) showing pronounced induction of theta frequency activity on perfusion with KA and loss of theta-band activity in the presence of CBX. (Cii) Power spectrum (300 s raw data obtained from same experiment as depicted in Ci). Scale bars, 250 s and 25 \( \mu \)V (Ai), 1 s and 20 \( \mu \)V (Bii and Ci).
total broadband power throughout the trial (mean total power was 268.0 nV²/Hz during control and 268.6 nV²/Hz during KA perfusion; n.s., n = 18). Area power within the theta frequency range (3–9 Hz) was significantly increased by KA; mean area power 3–9 Hz was 18.00 ± 2.59 nV²/Hz (10.85 ± 1.98% of total power; range, 3.97–20.03%) during control and 34.37 ± 6.97 nV²/Hz (15.62 ± 2.16% of total power; range, 5.30–36.42%) after 60 min of KA perfusion; P < 0.05, paired Student’s t-test, n = 18; Fig. 1A and B. This increase was partially reversed following 1 h wash. In three slices spontaneous theta-band activity was evident during the control period; this was inhibited by perfusion with KA (data not shown).

Inclusion of the gap junction blocker CBX (100 μM) and spironolactone (1 lM) reversibly blocked the effect of KA perfusion in four of five slices tested: during control, mean area power 3–9 Hz was 26.89 ± 6.92 nV²/Hz (9.64 ± 3.57% of total power); following 60 min KA perfusion it was 80.02 ± 22.76 nV²/Hz (23.51 ± 4.78% of total power); following 90 min KA, CBX and spironolactone perfusion (KA + CBX) it was 19.93 ± 5.80 nV²/Hz (5.75 ± 1.63% of total power); P < 0.05, ANOVA, n = 4; Fig. 1C. Spironolactone did not inhibit theta or gamma activity when applied alone (n = 3).

Slices maintained at room temperature (22–24°C) exhibited very low levels of spontaneous activity during control periods and no significant alteration in activity patterning within the theta frequency range on KA perfusion (mean area power was 5.60 ± 1.18 nV²/Hz during control and 5.36 ± 1.09 nV²/Hz following 60 min KA perfusion; n.s., n = 5, data not shown).

Perfusion with 10 μM DHPG evoked rhythmic LA oscillations within the gamma frequency band (20–40 Hz) in five of seven slices tested, mean area power 20–40 Hz was 46.93 ± 17.83 nV²/Hz during control (37.13 ± 7.57% of total power) and 96.61 ± 33.05 nV²/Hz at 60 min post-application (57.21 ± 5.01% of total power); P < 0.05, paired Student’s t-test, n = 7; Fig. 2A. Similar oscillations were also induced by perfusion with 50 μM carbachol (in three of five slices; data not shown). Oscillatory activity with DHPG was established within 40 min of the start of perfusion.

Inclusion of CBX and spironolactone with DHPG (DHPG + CBX; Fig. 2) evoked an alteration in the patterns of activity observed. Inhibition of gap junctions had no significant effect on total area power within the gamma frequency range (20–40 Hz): mean area power was 102.51 ± 5.10 nV²/Hz (59.82 ± 6.26% of total power) following 60 min DHPG perfusion and 107.55 ± 9.11 nV²/Hz (54.81 ± 5.92% of total power) at 90 min after application of DHPG + CBX (n.s., n = 4). However, it did evoke a shift in the modal peak (range 23.72–26.57 Hz during DHPG perfusion and 29.13–34.91 Hz during DHPG + CBX) and a significant reduction in the synchrony of activity within the LA, evident as an increase in the bandwidth of activity in the fast Fourier transform, the bandwidth of enhanced activity around the dominant modal peak being 1.5 ± 0.12 Hz in

![Fig. 2](image)

**Fig. 2.** DHPG induced gamma-band oscillations in the LA. (A) Unfiltered focal wave recording (i) during control and (ii) following induction of gamma-band activity induced by perfusion with 10 μM DHPG; note the presence of low levels of gamma activity during control and marked enhancement in amplitude during DHPG perfusion superimposed on a slow 0.5 Hz oscillation. (B) Power spectrum showing gamma-band activity induced by DHPG and the increased frequency and bandwidth of activity on inclusion of CBX; note that in this case there was little gamma frequency activity present during the control period. (C) Graph depicting average bandwidth of induced oscillatory activity in the presence of DHPG alone and DHPG with CBX; *P < 0.05, n = 4. Scale bars, 2 s and 10 μV (A).
DHGP and 2.68 ± 0.59 Hz in DHGP + CBX; P < 0.05, n = 4; Fig. 2B and C.

Discussion

Data presented here shows that oscillatory activity can be induced in the LA pharmacologically. Activity was induced at frequencies similar to those described in the LA in vivo (Paré & Collins, 2000; Collins et al., 2001). To our knowledge, this is the first demonstration of pharmacologically induced theta frequency activity within the LA in vitro. KA has been shown to induce theta frequency activity within the diagonal band of the medial septum (Garner et al., 2005), and is commonly used to induced gamma frequency activity in the hippocampus and neocortex. Interestingly, it is suggested that the gamma oscillatory activity usually observed within the hippocampus is superimposed on background oscillations within the theta frequency range (Fisahn et al., 1998; Fischer et al., 2002).

Similar to hippocampal studies (e.g. Pálhalmi et al., 2004), data presented here show induction of dominant gamma frequency activity within the LA on perfusion with either DHGP or carbachol. Gamma oscillations have been described in the amygdala following arterial perfusion of carbachol, in the isolated brain preparation (van der Linden et al., 1999). The band frequency of activity here parallels that described in the amygdala (20–35 Hz; Collins et al., 2001, van der Linden et al., 1999), slightly lower than that observed in the hippocampus and other neocortical regions. This probably reflects the complex unstratified cytoarchitecture of the amygdala nuclei, or reduced levels of drive, due to truncation of these pathways during slice preparation. This may reduce the strength of recurrent inhibitory pathways, thought to be vital for modulation of gamma and high-frequency ripples (Traub et al., 2003; Traub et al., 2004).

In contrast to hippocampal studies, gap junction blockade with CBX did not eliminate gamma frequency activity; however, the rhythmicity within this range was disrupted. This phenomenon is consistent with the view that gap junctions act as low-pass filters (Bennett & Zukin, 2004), as data presented here show loss of activity in the theta-band frequency on blockade of junctional activity. Studies show that desynchrony is required to maintain high-frequency activity (Netoff & Schiff, 2002); thus a reduction in gap junction activation should, theoretically, lead to an increase in frequency and disruption in the levels of rhythmicity, as observed here. Alterations in frequency dominance may also reflect the different forms of coupling present, e.g. axo-axonal coupling is linked to the propagation of high-frequency activity (Draguhn et al., 1998). Consistent with this hypothesis, a recent study has shown predominantly dendro-dendritic coupling of parvalbumin-positive GABAergic neurons in the basolateral amygdala (Muller et al., 2005). As it is suggested that GABAergic interneuron activity underpins neuronal activity pattern- and synchrony (Wang & Buzsaki, 1996; Whittington & Traub, 2003), it is feasible to suggest that, within the amygdala, electronically coupled interneurons play a strategic role in generation and maintenance of theta frequency activity thought to underlie neuronal processing during emotional perception. Furthermore, coupled networks may also play a pivotal role in controlling synchrony of neuronal activity at higher frequencies (Traub et al., 2003), linked to cognitive and perceptual processing.

In addition, the subunit composition of GABAergic neurons may alter the dynamics of network activity, through modulation of channel kinetics. Indeed, in the hippocampus, GABA_A receptors containing the δ subunit have been shown to modulate oscillatory activity within the gamma frequency range (Towers et al., 2004), and subunit composition has also been implicated in the regulation of high-frequency oscillations (Ponomarenko et al., 2004). Differential expression of GABA subunits has been demonstrated within the amygdala nuclei and has been linked to the variability in response to various anxiolytic drugs (Fujimura et al., 2005). In comparison with the hippocampus, the amygdala shows a significantly higher level of expression of the α1–3 and β1 and 2 subunits and greatly reduced expression of α5 (Pirker et al., 2000). Thus, differences in the subunit composition of GABAergic networks may provide another mechanism by which intra-amygdala activity can be sculpted and also underlie differences in frequency profiles between regions.

In these ways, patterning of activity through the intra-amygdala circuitry can be modulated and integrated in a plastic manner. As this capacity is maintained in the in vitro preparation it suggests that the generation of this activity is intrinsic to the intra-amygdala circuitry, but this does not exclude external derivation, e.g. via thalamocortical or septohippocampal pathways. As such, the in vitro model described provides a useful tool for dissecting the components comprising the generation and modulation of these activity patterns.

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Abbreviations

DHGP, (RS)-3,5-dihydroxyphenylglycine; aCSF, artificial cerebrospinal fluid; CBX, carbonoxolone; KA, kainic acid; LA, lateral amygdala.

References


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