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Short Term Exposure to Ketamine Reduces Cholinergic Synaptic Transmission, But Not Post-Tetanic Potentiation in Central Neurons

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Abstract

The NMDA receptors are thought to be the main target site for the actions of dissociative ketamine. In addition, ketamine has also been shown to affect myriad brain regions and functions, including learning and memory. However, the precise target sites and the mechanisms underlying ketamine actions in the mammalian central nervous system (CNS) remain poorly defined, mainly due to anatomical constraints, and our inability to access individual pre and postsynaptic neurons. Within the CNS of *Lymnaea*, the neurons visceral dorsal 4 (VD4) and left pedal dorsal 1 (LPeD1) form an excitatory cholinergic synapse which recapitulates reliably *in vitro*.

Cell culture and electrophysiological techniques were used in this study to determine the effects of ketamine on synaptic transmission and post tetanic potentiation (PTP) at the VD4-LPeD1 synapse.

Acutely applied ketamine significantly decreased synaptic transmission between VD4 and LPeD1, although not in a concentration dependent manner; it did not however significantly affect short-term synaptic plasticity which is thought to underlying short-term, working memory.

This work provides direct evidence that ketamine reduces cholinergic synaptic transmission, but that it does not compromise neuronal synaptic plasticity between central neurons.

Keywords: *Ketamine; Lymnaea stagnalis; Acetylcholine; synaptic transmission*

Abbreviations: 5-HT: 5-hydroxytryptaline; ABS: Antibiotic saline; Ach: Acetylcholine; ANOVA: Analysis of variance; CAMKII: Ca^{2+}/calmodulin-dependent protein kinase 2; CM: Conditioned media; CNS: Central nervous system; DA: Dopamine; DM: Defined media; EC_{50}: Half maximal effective concentration; EPSPs: Excitatory postsynaptic potentials; HCL: Hydrochloride; HODM: High Osmolarity Defined Medium; IV: Intravenous; LPeD1: Left pedal dorsal 1; LTP: Long term potentiation; NA: Noradrenaline; nAChR: Nicotinic Acetylcholine Receptor; NGF: Nerve growth factor; NMDA : N-methyl-d-aspartate receptors; NMJ: Neuromuscular junction; PTP: Post tetanic potentiation; SEM: Standard error of the mean; SPSS: Statistical Package for the Social Sciences; VD4: Visceral dorsal 4;

Introduction

ANESTHETICS are required for the induction and maintenance of anesthesia, which is prerequisite to all major surgical procedures. However, despite recent progress the precise sites of action and the underlying mechanisms remain unknown. Moreover, recent evidence strongly supports the notion that various inhalation anesthetics impair memory and that even a brief exposure in children and elderly may impart long-term harm *vis-à-vis* cognition, learning and memory deficit [1-4]. Similar evidence for local anaesthetics affecting neuronal communications between central neurons is however lacking. Because the cholinergic pathway plays essential roles in learning and memory, it is therefore imperative to determine whether local anesthetics affect a) synaptic transmission and b) synaptic plasticity between individual pre-and postsynaptic neurons.

Racemic ketamine was approved for use in clinical procedures in 1970 [5] as its enables short duration, painful surgical procedures as the sole analgesic and anesthetic agent [6]. Ketamine has been widely used in paediatric anaesthesia [7] and is often used for induction of anaesthesia for caesarean section [8]. There is a vast array of literature available regarding the effects of clinical doses of ketamine, its varied routes of administration and uses. Doses range from 0.5 to 10 mg/Kg, which equates to between 2.1 and 42 μM. Administration can be via intravenous, oral or intramuscular routes, for anaesthesia, pain relief and even asthma [9,10]. Ketamine is also abused widely as a recreational drug, in an uncontrolled acute and sometimes chronic manner. Research extending above the clinical range must be carried out in order to establish the detrimental effects this anesthetic may have, and future implications for individuals abusing it. Here we used acute doses of 10, 25, 50 and 100 μM Ketamine in order to represent both clinical and abused levels of ketamine.

Although it is commonly accepted that many of the responses relevant to anesthesia are mediated via the NMDA receptor, the potential targets for ketamine are diverse and include both voltage and ligand operated channels and reuptake processes of monoamines such as noradrenaline (NA), dopamine (DA) and 5-hydroxytryptamine (5-HT) [11]. Ketamine has also been shown to have some affinity for nicotinic [12] and muscarinic cholinergic [13], dopaminergic [14] and GABAergic receptors [11]. Morgan and Curran [15] suggested that the variability in results was due to the differing doses used in various studies, and the use of other interacting drugs taken by subjects.

The presynaptic and postsynaptic effects of ketamine at the neuromuscular junction have also been investigated. In the frog, ketamine has been shown to interact with open ACh activated ion channels, thus blocking neuromuscular transmission postsynaptically [16]. This work was supported by that of Wachtel [17] which showed a reduction in opening times of ACh activated channels. Savage & Sanya [18] found nicotine, carbachol and electrically induced contractions were facilitated by ketamine at low concentrations and inhibited at higher concentrations and this biphasic response had been demonstrated previously by Torda & Murphy [19] upon presynaptic release at the mouse neuromuscular junction.

Learning and memory have been shown to be affected by anesthetics including ketamine. During anaesthesia, memory can be classified as explicit and implicit, and the effects of both inhalation and IV 'anesthetics' have been investigated with regards to loss of both types of memory [20,21]. These investigations are however limited by the complex nature of the mammalian nervous system, where investigation of the isolated synapse is not currently possible. Short-term memory manifests itself as short-term plasticity which is an integral part of the functioning of the nervous system. Learning and memory have been shown to be affected by anesthetics including ketamine. PTP is a short term facilitation of synaptic efficacy lasting tens of seconds to minutes, which follows presynaptic tetanic activity and is important for the formation of memory. In vertebrates PTP has been investigated in the frog [22] and rat NMJ [23] and in hippocampal synapses [24]. In invertebrates PTP has been demonstrated in the crayfish [25] and *Aplysia* NMJ [26] as well as *Helix* [27,28] *Aplysia* [29] and *Lymnaea* central synapses [30]. These data demonstrate the variability of mechanisms of PTP at synapses in the same preparation; however there are two common features of PTP in all preparations: PTP requires both presynaptic tetanic activity, and decays in a graded manner with time.

In this study, the effects of the dissociative anesthetic ketamine ($C_{13}H_{16}ClNO$) were investigated at an identified, excitatory, cholinergic synapse from *Lymnaea*. Ketamine is an arylcyclohexylamine [31], known in mammals to bind to the phencyclidine binding site

located within the N-methyl-D-aspartate (NMDA) receptor. Here, we provide the first direct evidence that ketamine affects synaptic transmission and synaptic plasticity in the form of PTP, without the confounding factors experienced by previous studies.

Materials and Methods

Animals

Laboratory bred specimens of the freshwater snail *Lymnaea* were maintained at room temperature in dechlorinated, well aerated water and fed lettuce. Specimens aged 1-2 months (measuring approximately 1-1.5 cm shell length) were used for cultured electrophysiology. For production of conditioned medium (CM) more mature snails, aged 2-4 months (measuring 1.5-2.5 cm) were used.

Cell Culture

Isolation and culture of cells was carried out as described previously [32]. All experiments were carried out under sterile conditions using a culture hood and solutions sterilised by autoclave or filter sterilisation using 0.2μm filters where appropriate. In summary snails were anesthetized in a ten percent solution of Listerine (ethanol 21%, menthol 0.042%) in tissue culture HEPES saline (composition; Na^{2+} 40 mM, Cl- 52.9 mM, K^{+} 1.7 mM, Mg^{2+} 1.5 mM, Ca^{2+} 4.1 mM, HEPES 10 mM pH 7.9). After transfer into antibiotic HEPES saline -ABS (tissue culture HEPES saline containing; 225 μg/ml gentamicin sulphate) the brain was removed as described previously [32] and then washed three times for 5 minutes in ABS. The ganglia were then soaked in a 2 mg//ml trypsin solution (Sigma) for 23 minutes followed by a 2 mg/ml trypsin inhibitor solution (Sigma) for 15 minutes. Trypsin solutions were made in defined media - DM (Life Technologies, Gaithersburg MD: special order - composition; Liebowitz15 Glutamax media, salts, 25 μg/ml gentamicin sulphate).

The brains were transferred finally to high osmolarity defined medium (HODM) (composition; DM, 15mM glucose) in a dissection dish with and pinned out to allow removal of cells from the brain. Cells were removed by applying gentle suction via a fire polished, Sigmacote (Sigma) treated pipette attached via tubing to a Gilmont micro barrel burette (CP instrument Co.UK) and filled with HODM. The cells were then plated in Falcon 3001 dishes (Beeton Dickinson, France) modified for culture and coated in a 0.1% poly-L-lysine (Mwt 111,000- Sigma) Tris buffered solution (Aldrich, UK). Cells were grown in CM (produced by incubating whole brains in DM to extract NGF from the whole brain, see [32] Syed., *et al*. for detailed method) in an incubator at 20°C. For formation of soma-soma connections the cells were removed without axon attached and plated directly next to each other, cells were incubated overnight and recordings made the following day.

Electrophysiological Recording

Standard electrophysiological techniques were used to monitor neuronal activity [33]. Simultaneous recordings were made from VD4 and LPeD1 before during and after perfusion with ketamine, and single EPSPs were generated by electrically stimulating the presynaptic cell to fire an action potential. Specifically, filamented glass microelectrodes (internal diameter 1.5 mm, World Precision Instruments, Sarasota, FL) were pulled to a tip resistance of 20-40 MOhms and filled with a saturated solution of K_2SO_4. The cultured neurons were viewed using an inverted microscope (Axiovert 135, Zeiss, Thornwood, NY), perfused with tissue culture HEPES saline (composition; Na^{2+} 40 mM, Cl- 52.9 mM, K^{+} 1.7 mM, Mg^{2+} 1.5 mM, Ca^{2+} 4.1 mM, HEPES 10mM, pH 7.9) and impaled using Narishige (Tokyo, Kapan) micromanipulators. Intracellular electrical signals were amplified using a Neurodata IR283 amplifier (Neurodata Instrument corporation), and displayed using an oscilloscope (Tektronix Inc., Oregon USA), an Apple Macintosh computer (axograph chart software) and Gould chart recorder (TA 240S, Gould, Cleveland, Ohio). During experiments assessing the effect of ketamine on short term potentiation experiments EPSPs were followed by tetanic stimulation of the presynaptic cell, generating a compound EPSP in LPeD1, this was then followed by four individual EPSPs being generated. This was carried out before during and after perfusion with ketamine.

Drugs

Ketamine HCl (Parke Davis) was diluted from a sterile stock solution to 10, 25, 50 and 100 μM and perfused *via* the flow system. AChCl (10mM, Sigma pharmaceuticals) was applied *via* picoinjection.

Data analysis

For experiments recording changes in EPSP amplitudes, data is represented as the mean amplitude ± SE (mV). Data was analysed using Axograph chart software package, graphs were constructed using Microsoft Excel and SPSS was used for statistical analysis. Student's t-tests were carried out to assess the significance of data, followed by one-way ANOVA where appropriate. Statistical significance was calculated using 95% confidence interval ($P < 0.05$). Analysis of data recorded in experiments monitoring changes in PTP was carried out as follows, the pretetanic EPSP (control EPSP) was normalised to 100% and all other EPSP amplitudes were then expressed as a percentage of the control ± SE. Data were analysed using Axograph chart software package, graphs were constructed using (Microsoft) Excel and SPSS was used for statistical analysis. Student's t-tests were carried out to assess the significance of data, followed by one-way ANOVA where appropriate. Statistical significance was calculated using 95% confidence interval ($P < 0.05$).

Results

Ketamine affects excitatory cholinergic neurotransmission between identified neurons

In vivo, the neuron VD4 forms a unidirectional synapse with its postsynaptic partner LPeD1 and this cholinergic synapse is recapitulated when cells are paired in vitro either in a neurite-neurite or the soma-soma configuration [34,35]. When cultured in the presence of brain-conditioned medium (CM) either in an axon-axon (Figure 1A) or soma-soma configuration (Figure 1C) neurons established their specific excitatory connections which were similar to those seen in vivo. For instance, simultaneous recordings revealed that action potentials in VD4 generated1:1 action potentials (Figure 1B) or elicited 1:1 excitatory postsynaptic potentials (EPSPs –Figure 1D) in LPeD1 (n = 24,100%).

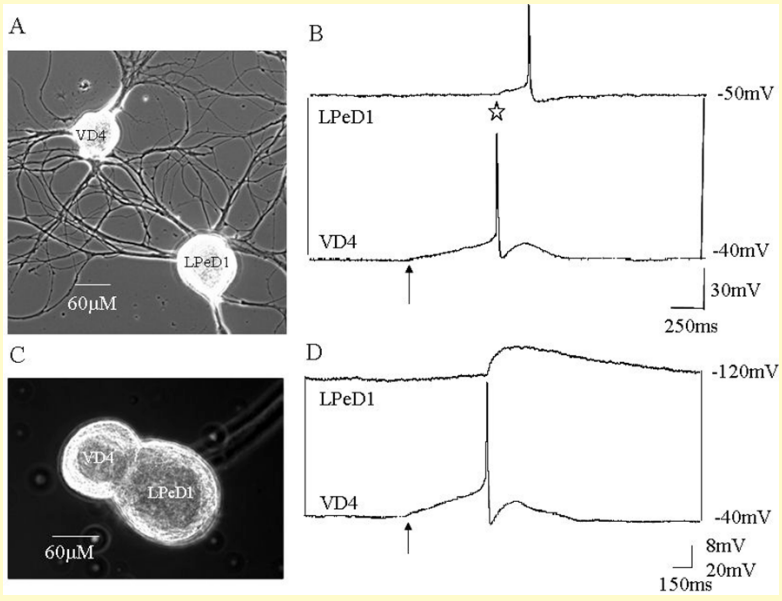

Figure 1: *VD4 and LPeD1 form neurite-neurite synapses (A), and soma-soma synapses (C) when paired overnight. In soma-soma configuration intracellular depolarizing pulses delivered to VD4 (at arrows) resulted in an action potential (B-star) at resting membrane potential, or an EPSP at the hyperpolarizing potential of -120 mV (D) in LPeD1 (n = 24,100%).*

To test whether ketamine affects cholinergic transmission between identified neurons VD4 and LPeD1, cells were soma-soma paired overnight. Specifically, individually isolated neurons were juxtaposed in cell culture and left overnight. On day two, simultaneous intracellular recordings were made and synapses were tested electrophysiologically using fast perfusion system [34] to apply control

saline or clinically relevant concentrations of ketamine dissolved in normal saline. Electrically induced action potentials in VD4 generated 1:1 EPSPs in LPeD1 held at the hyperpolarizing potential of –120mV (Figure 1D). Ketamine at concentrations of 10 μM, 25 μM, 50 μM and 100 mM (Figure 2B-D) significantly reduced the amplitude of VD4-induced EPSPs in LPeD1 ($p < 0.05$). It is also important to note that larger currents were often required to trigger action potential in VD4 maintained in the presence of ketamine. This synaptic transmission however recovered only partially upon wash out with normal saline even when the preparation was maintained in normal saline for ten minutes of observation time (Figure 2A-E).

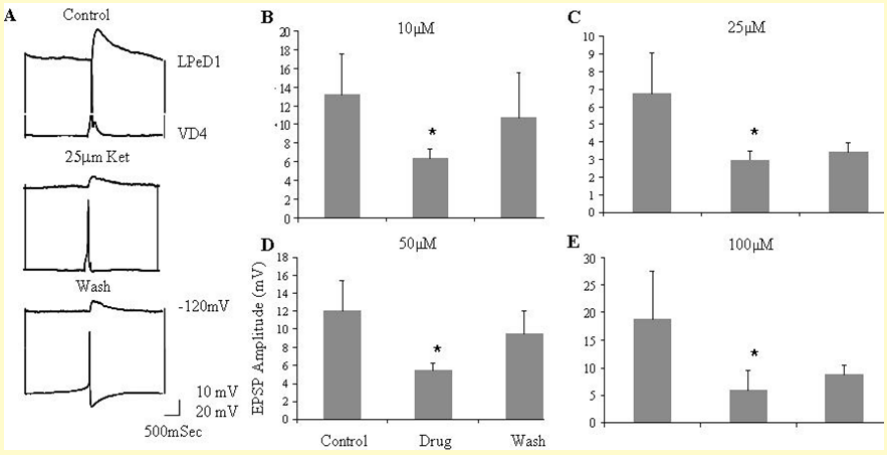

Figure 2: *(A) VD4 (normal resting potential) was stimulated electrically to fire an action potential, which resulted in an EPSP in LPeD1 (A), this was used as control data. Clinical doses of ketamine (50 μM shown) suppress synaptic transmission between LPeD1 and VD4 (B), however recovery after ten minutes was incomplete. Mean EPSP amplitude (mV ± SEM) was significantly (*) reduced in the presence of ketamine (10, 25, 50 and 100 μM) compared with control ($p < 0.05$, $n = 4$), however recovery was incomplete (B-E).*

Exposure to 10 μM ketamine (n = 4) decreased mean EPSP amplitude by 7.41mV from 13.73 ± 4.32 mV to 6.32 ± 1.06 mV. Upon washout with normal saline, the EPSPs amplitude recovered to 10.73 ± 4.88 mV (Figure 2B). Similarly, 25 μM ketamine (n = 4) reduced EPSP amplitude from 6.74 ± 2.30 mV to 2.96 ± 0.50 mV, a decrease of 3.78 mV. The recovery was also partial (3.41 ± 0.53 mV, Figure 2C). Treatment with 50 μM ketamine (n = 5) on the other hand, decreased the EPSP amplitude by 6.59 mV from 11.99 ± 3.45 mV to 5.40 ± 0.81 mV. After washout with normal saline, only partial recovery of the EPSP amplitude was observed (9.53 ± 2.52 mV – see Figure 2D). Similarly, 100 μM ketamine (n = 4) decreased the EPSP amplitude from 18.76 ± 8.80 mV to 5.93 ± 3.61 mV, a reduction of 12.83 mV. Washout resulted in a recovery of only 2.80 mV to a mean EPSP amplitude to 8.73 ± 1.68 mV (figure 2E). Statistical analysis showed no significant difference between various concentrations ($p < 0.05$). Taken together, the above data demonstrate that clinically relevant concentrations of ketamine diminish synaptic transmission between Lymnaea neurons, although not in a dose-dependent manner. However, the recovery from this suppression was only partial for all concentrations tested within a short-term specified time. It is important to note that all experiments were carried out at the fixed membrane potential of -120 mV, however it was noted that application of ketamine brought about some level of hyperpolarisation of LPeD1, this was not however investigated any further.

Ketamine did not affect short term potentiation in *Lymnaea*

To establish the nature of synaptic potentiation at the VD4 LPeD1 synapse, simultaneous intracellular recordings were made from the pair. Induced action potentials in VD4 generated 1:1EPSPs (at open arrow see Figure 3A). A tetanus was then elicited in VD4 (depolarising current 6-10 action potentials) which resulted in a compound EPSP in LPeD1, or occasionally generated action potentials in the

postsynaptic cell (at closed arrow see Figure 3A). Subsequently a single action potential fired in VD4 resulted in a potentiated response in LPeD1 and the resulting EPSPs reached threshold and triggered spikes in the postsynaptic cell (at star Figure 3A).

This novel form of PTP is thought to form the basis of working memory and was demonstrated to be use rather than time-dependent [37]. To establish whether short-term potentiation at the VD4-LPeD1 synapse is time or use dependent, the soma-soma pairs were prepared overnight and simultaneous intracellular recordings were made. Induced action potentials in VD4 generated 1:1EPSPs which after the tetanic stimulation exhibit synaptic potentiation as described above and synaptic transmission returned to its base line after either 2-4 actions potentials in VD4 (see Figure 3B). To test the use-dependency of this response, after delivery of tetanus in VD4, the cell was kept hyperpolarized for several minutes. After several minutes of lapse time, VD4 was induced to fire an action potential. This single action potential, regardless of the time that it was delivered, generated a potentiated response in LPeD1 (Figure 3c). These data confirm a previous study that synaptic potentiation between VD4 and LPeD1 is use but not time dependent [36]. This potentiation was clearly seen when LPeD1 was hyperpolarised, and the size of the resulting EPSP measured (Figure 3D). The data was normalised to the pretetanic EPSP amplitude and expressed as % potentiation. The amplitude of the first post tetanic EPSP ($n = 14$, $p< 0.05$) was found to be significantly larger than the mean pre tetanic EPSP, increasing by 235.5 % (to 335.5 ± 31.2 %, Figure 3D), the amplitude of the second post tetanic EPSP was also significantly increased (by 174.7 % to 274.7 ± 31.2 %, $n = 14$). By the third EPSP, although still increased, the amplitude was returning to pre tetanic levels (142.9 ± 16.7 %, n=10). The potentiated response however, returned to its pre-tetanic level by the fourth EPSP (108.09 ± 16.71 %, $n = 5$, Figure 3D). These data confirm previous observations that the VD4-LPeD1 synapse exhibits short-term plasticity which is thought to account for working memory.

Post tetanic potentiation was assessed either before, during or after Ketamine pre-treatment of the soma-soma paired cells via the fast flow perfusion system [36] Action potentials in VD4 generated EPSPs in LPeD1 which potentiated after tetanus. The controls for each of the 4 doses (total $n = 14$) of ketamine resulted in consistent potentiation of the first post-tetanic EPSP to around 200% (Figure 4A-D). After switching solution to 10 μM ketamine, potentiation of the post tetanic EPSP increased by 31.3 mV (from 180.6 ± 16.0 % to 211.9 ± 42.1 %, $n = 4$). These effects were however statistically insignificant ($p< 0.05$) compared with control. Similarly, the amplitude of the second EPSP was also enhanced from 126.1 ± 21.6 % to 148.4 ± 27.1 %, but this increase was also statistically insignificant (Figure 4A, $p< 0.05$) compared with control. 25 μM ketamine (n=5) also had no significant effect on PTP, with the first post tetanic EPSP been changed only 7.9 % from 179.9 ± 33.6 % to 171.9 ± 26.7 %, in the presence of ketamine and no significant difference after washing (Fig. 4B, <0.05). Similarly, neither 50 μM ($n = 5$, Figure 4C) nor 100 μM (n=4, Figure 4D) ketamine significantly increased PTP (increases from 221.5 ± 23.5 to 238.7 ± 23.1 % and 234.9 ± 25.6 % to 245.5 ± 24.4 respectively, $p< 0.05$). After treatment with 50 μM and subsequent washout, the amplitude of post tetanic EPSP however decreased from the control value of 221.5 ± 23.5 to 87.4 ± 28.2 %. No difference between control and washout was noted when treated with 100 μM, however it must be noted that the variability of this data was noticeably increased (see Figure 4D). These data demonstrate for the first time that ketamine does not affect PTP at an identified excitatory cholinergic synapse in Lymnaea.

Discussion

This study has demonstrated that ketamine blocks cholinergic excitatory synaptic transmission, and provides direct evidence that neither the expression of short term plasticity nor its retention were affected. The wide distribution of nAChRs in the CNS, peripheral nervous system and also at NMJs [37] indicates their involvement in a wide variety of processes and implies their involvement in cognitive performance, nociception [38] and psychoneurological disorders such as Alzheimer's and Parkinson's diseases [39].

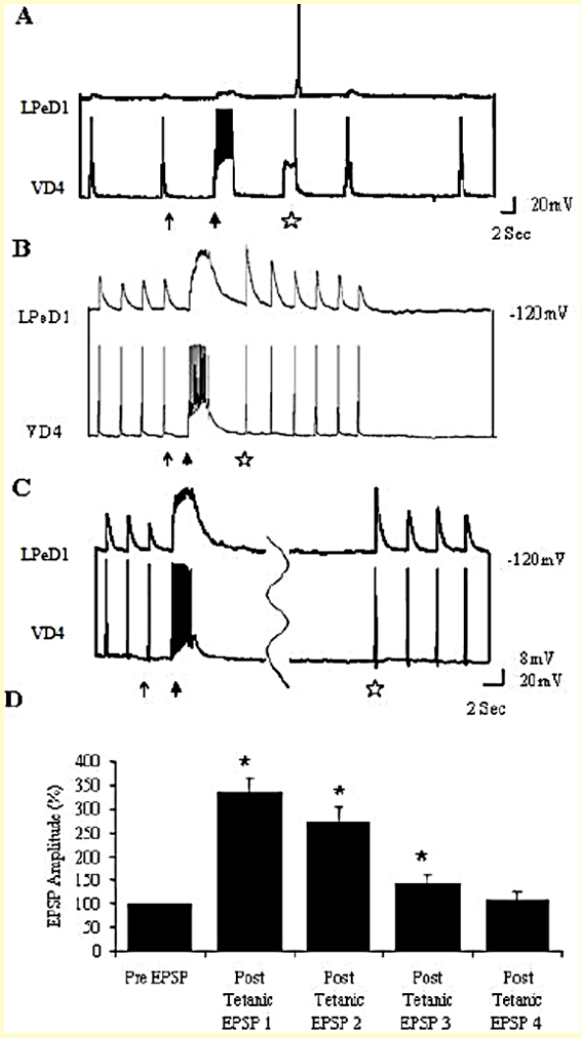

Figure 3: *Generation of EPSPs in LPeD1 by stimulation of VD4 (arrows), followed by tetanic stimulation in VD4, results in a compound EPSP in LPeD1 (arrowheads). This compound EPSP is then followed by a potentiated response in LPeD1, which results in an action potential (A-star) when the cell is close to threshold, or a potentiated EPSP below threshold (B-star). This potentiated response returns to control levels within 4 or 5 EPSPs (B).*

After a time delay of approximately 15 minutes, this compound EPSP is still followed by a potentiated response in LPeD1 (C-star), which returns to control levels within 4 or 5 EPSPs. (D) Pre EPSP amplitude was normalised to 100%, and the post tetanic EPSP amplitudes expressed as % EPSP (mV, mean ± SEM). Post tetanic EPSP1 was significantly (*) increased compared to control ($p < 0.05$, $n = 14$), as was post tetanic EPSP2 ($p < 0.05$, $n = 14$) and post tetanic EPSP3 ($p < 0.05$, $n = 10$). The fourth post tetanic EPSP was not however significantly different from the control. ($p < 0.05$, $n=5$).

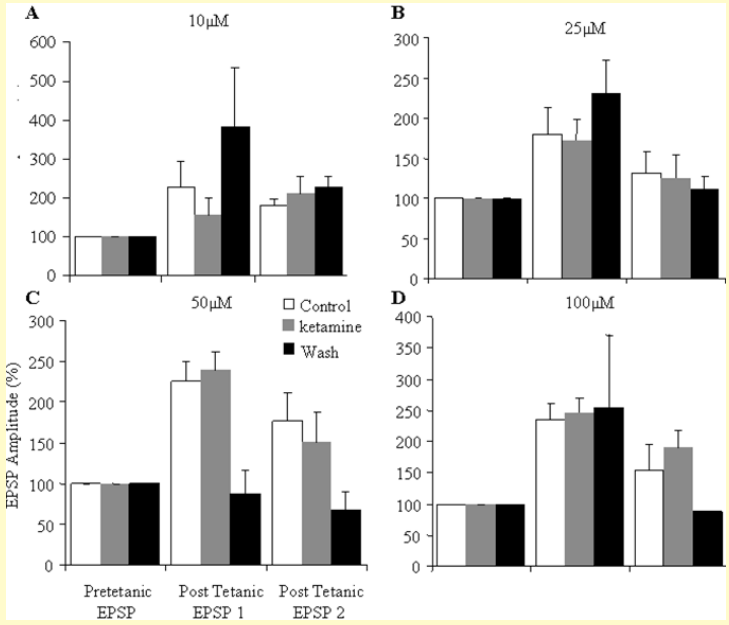

Figure 4: *Control values were normalised to 100% (n = 14), and drug and wash values were expressed as mean % increase (mV ± SEM). Ketamine (10, 25, 50 and 100μM) did not significantly increase post tetanic EPSP 1 or 2 amplitude compared with control (p< 0.05, n = 4,4,5,4 respectively).*

Relatively little is known however about the cellular mechanisms by which ketamine affects cholinergic synaptic transmission between the central neurons, and whether these are relevant to anaesthesia. In 1982 Aronstam., *et al*. found ketamine to inhibit cholinergic transmission at similar doses to those used in this study [40] – albeit not at the level of single pre-and postsynaptic neurons. In the present study an in vitro approach was used to determine whether acutely applied concentrations of ketamine bind to AChR blocking synaptic transmission in a dose dependent manner, and whether these effects were reversible. It is important to note that a variety of nAChR have been cloned from *Lymnaea* and they exhibit significant homology to their mammalian counterparts [41].

In this study VD4 and LPeD1, formed synapses and maintained electrical properties when cultured in the presence of brain-conditioned medium (CM) either in an axon-axon or soma-soma configuration. The responses obtained were similar to those shown previously *in vivo*, where VD4 forms a unidirectional synapse with its postsynaptic partner LPeD1, and the work of [35,42] demonstrated that this synapse is recapitulated when cells are paired *in vitro* either in a neurite-neurite or soma-soma configuration.

Plasma concentration of ketamine in clinical settings has been determined to be 10 μM during general anaesthesia after IV injection [43,44], and EC_{50} values of 4 μM [44], 7 μM [45], 5 μM [43], and 15 μM [46] have been noted. The above data support the hypothesis that a clinically relevant concentration of ketamine blocks synaptic transmission between *Lymnaea* neurons – albeit not in a dose dependent manner. Moreover, the recovery from this suppression was only partial for all concentrations tested.

The effects of 1-100 μM ketamine on another cell pair right pedal dorsal 1 (RPeD1) and VD4 were investigated by Woodall and McCrohan [47], where no effect on resting membrane properties were observed in either cell. Ketamine's mode of action is to reduce excitation, and as RPeD1 and VD4 mainly form a mutual inhibitory connection, transmission between the cells was not investigated in their paper. In this study however, within clinically relevant concentrations (10μM-100μM), ketamine significantly reduced the amplitude of VD4 induced EPSPs in LPeD1. It is also important to note that larger currents were often required to trigger action potentials in

VD4 maintained in the presence of ketamine [47]; Woodall & McCrohan did not however report any change in input resistance in VD4 during their investigation. Synaptic transmission recovered only partially upon wash out with normal saline even after 10 minutes.

Previous studies have shown that anesthetics affect memory [48] and demonstrated that during swim trials rats previously treated with ketamine did not learn as well as controls. Ketamine has been shown to decrease the occurrence and extent of LTP but not to completely block it [49], and in the hippocampus induction of LTP has also been shown to be inhibited [50]. It has been proposed that LTP is mediated by the NMDA receptor [51,52], and there is considerable animal model based evidence for both the role of NMDA in memory and the neurotoxicity of ketamine [53,54]. Unequivocal evidence for ketamine induced effects on short term potentiation has not yet been obtained, owing primarily to the complexity of the mammalian brain. Using *Lymnaea* neurons as a model system, a novel form of post tetanic potentiation has been demonstrated [30]. This potentiation persists from a few seconds to minutes and is a form of short term synaptic plasticity. There are many potential sites where ketamine could interfere with PTP, mainly postsynaptic receptor sensitivity and presynaptic transmitter release. Short term potentiation in *Lymnaea* has been shown to be due to increased transmitter release resulting from calcium-activated CAMKII [36].

Data in this investigation confirm previous observations that the VD4-LPeD1 synapse exhibits short-term potentiation, which is thought to account for working memory. Working memory is often thought to be use rather than time-dependent, and after several minutes of lapse time, VD4 was induced to fire an action potential which generated a potentiated response in LPeD1. These data thus demonstrate that the synaptic potentiation between VD4 and LPeD1 is use and not time dependent. After switching the normal bathing solution to that containing ketamine, potentiation of the post tetanic EPSP was not significantly different from control values, but it must be noted that the variability of this data was noticeably increased. These data demonstrate that ketamine does not significantly affect PTP at the *Lymnaea* excitatory, cholinergic synapse between VD4 and LPeD1.

General anesthetics may cause perturbation of neural function in both the young and the elderly. Studies on immature rats (7 days old), at the peak of brain development, demonstrated that general anesthetics may induce long-lasting ultrastructural changes at synapses in the subiculum, as well as permanent neuronal loss, both of which may contribute to learning deficits in later life [1]. Previous studies on young (6 months old) rats and aged rats (18 months old) revealed that anesthetization with isoflurane and nitrous oxide improved maze performance in the young rats, but attenuated the performance in older animals [2]. A meta-analysis of work on neonatal exposure of rodents and primates to general s indicates a correlation with neurotoxic effects including increased programmed cell death and neurocognitive deficits [3], but retrospective analysis of clinical data was inconclusive. However, an analysis of children repeatedly exposed to general anesthetics before the age of 2 years suggests that they are at increased risk of developing attention deficit hyperactivity disorder (ADHD) [4].

According to Crosby and Culley [55], several agents including ketamine can produce post-operative delirium in the elderly, although it is most strongly associated with anticholinergic agents such as atropine and scopolamine. However, ketamine is also known to have neuroprotective effects and anti-inflammatory actions [56] and has been shown to attenuate delirium [57] and postoperative cognitive function (POCD) [58] after cardiac surgery, but to have little influence on the occurrence of POCD in elderly patients undergoing orthopedic surgery. Two points arise from these observations. First the long term cellular actions will need substantial further study, particularly as we have demonstrated here that ketamine blocks synaptic transmission between cultured *Lymnaea* neurons, but there is only partial recovery following washout. Second, it is clear that different types of anesthetics act in very different ways [59,60] and many these differences can be elucidated at a cellular level using cultured, identified *Lymnaea* neurons.

Although we observed that ketamine blocks synaptic transmission, yet the ratio between pre- and post-tetanic potentials remains unaffected. One hypothesis would be that the pre-tetanic EPSP is depressed, and although tetanic bursting will result in intracellular release of calcium and more transmitter release, the postsynaptic receptors remain blocked and therefore the ratio will remain the same. i.e. presynaptic mechanisms of transmitter release are unaffected by ketamine. These results are supported by Naruo., *et al*. [61]

who demonstrated that sevoflurane blocked cholinergic transmission postsynaptically between VD4 and LPeD1, but did not affect short term potentiation, and concluded that this was due to the presynaptic mechanisms involved in PTP in *Lymnaea*.

Conclusions

Using an excitatory cholinergic synapse between identified, cultured neurons from the mollusc Lymnaea stagnalis, we have shown that acute administration of ketamine, at both clinical and abused levels of concentration, significantly decreases synaptic transmission, although not in a concentration dependent manner. Using the same preparation we have also demonstrated, for the first time, that ketamine does not significantly affect short term synaptic plasticity in the form of post-tetanic potentiation. This suggests that ketamine may be acting postsynaptically, but further experiments will be necessary to test this assertion.

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