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Effects of Microinjections of Group II Metabotropic Glutamate Agents into the Amygdala on Sleep

Enheng Dong, Ph.D., Laurie L. Wellman, Ph.D., Linghui Yang, M.S., and Larry D. Sanford, Ph.D.

Sleep Research Laboratory, Department of Pathology and Anatomy, Eastern Virginia Medical School, Norfolk, VA, USA

Abstract

Systemic administration of the group II metabotropic glutamate (mGlu) receptor agonist, LY379268 (LY37), dose-dependently suppresses rapid eye movement sleep (REM) whereas systemic administration of the mGlu II receptor antagonist, LY341495 (LY34), increases arousal. Group II mGlu receptors are highly expressed in the amygdala, a brain region involved in the regulation of sleep and arousal. To determine whether the amygdala is involved in mediating the effects of Group II mGlu agents on sleep, we microinjected LY37 and LY34 into the basal amygdala (BA) and the central nucleus of the amygdala (CNA) and recorded sleep and wakefulness. Wistar rats were implanted with electrodes for recording sleep and with bilateral cannulae aimed into BA for drug administration. Different groups of rats received bilateral microinjections of LY37 into BA at two dosage ranges (3.2 mM, 5.3mM or 10.7 mM or 0.1 nM, 2.0 nM or 10.0 nM) or one dosage range of LY34 (1.0nM, 30.0nM or 60.0nM). Microinjections into CNA were conducted at one dosage range for LY37 (0.1 nM, 2.0 nM or 10.0 nM) and for LY34 (1.0nM, 30.0nM or 60.0nM). All drugs or vehicle alone were administered in a counterbalanced order at 5-day intervals. Following microinjection, sleep was recorded for 20 h. Microinjection of LY37 into BA at both nM and mM concentrations significantly decreased REM without significantly altering NREM, total sleep or wakefulness. The high dosage of LY34 in BA significantly suppressed NREM and total sleep. Microinjections of LY37 or LY34 into CNA had no significant impact on sleep. We suggest that Group II mGlu receptors may influence specific cells in BA that control descending output (via the CNA or bed nucleus of the stria terminalis) that in turn regulates pontine REM generator regions.

Keywords

amygdala; metabotropic glutamate; rapid eye movement sleep; sleep

1. Introduction

Glutamate is the major excitatory neurotransmitter in the brain and it plays an essential role in regulating arousal (Jones, 2005). It acts through two major groups of receptors: ionotropic

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Address correspondence and requests for reprints to: Larry D. Sanford, Sleep Research Laboratory, Department of Pathology and Anatomy, Eastern Virginia Medical School, P.O. Box 1980, Norfolk, VA 23507., TEL:(757) 446-7081, FAX:(757) 446-5719, SanforLD@evms.edu.

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glutamate (iGlu) receptors and metabotropic glutamate (mGlu) receptors. Eight types of mGlu receptors have been cloned, which are classified into three groups (I, II, III) according to their sequence homology, second messenger mechanism and pharmacologic activity (Palucha and Pilc, 2007). mGlu II (mGlu2 and mGlu3) receptors are located mainly outside the active zone area of presynaptic terminals. They may only be activated in instances of significantly enhanced glutamate release and are thought to act as autoreceptors to inhibit glutamate release and restore normal levels (Cartmell and Schoepp, 2000; Schoepp, 2001). This potential for regulating glutamate has led to interest in mGlu II receptors as therapeutic targets for the treatment of anxiety and stress-related disorders (Swanson et al., 2005). In fact, mGlu II agents are effective in reducing anxiety in a number of preclinical models (Johnson et al., 2005).

Work at the systemic level has implicated mGlu II receptors in sleep regulation. Subcutaneous injection of the mGlu II receptor agonist, LY379268 (LY37), induced a dose-dependent suppression of REM (Feinberg et al., 2002). In addition, subcutaneous injection of the mGlu II agonist, LY354740 (LY35), dose-dependently suppressed REM and prolonged its onset latency in both rats and mice but did not alter REM in mGlu2 receptor negative mice (Ahnaou et al., 2009). Moreover, systemic administration of the mGlu II receptor antagonist, LY341495 (LY34), increased arousal by suppressing both REM and NREM (Feinberg et al., 2005). However, the potential neural site(s) that mediate these changes in sleep has not been determined.

mGlu II receptors are expressed in the amygdala (Ohishi et al., 1993a; Ohishi et al., 1993b) (Gu et al., 2008; Tamaru et al., 2001), a limbic structure increasingly implicated in the regulation of arousal and sleep (Deboer et al., 1999; Jha et al., 2005b; Morrison et al., 2000; Sanford et al., 1998; Sanford et al., 2002; Tang et al., 2005) as well as having a demonstrated role in emotion and anxiety (Davis, 1997; Davis and Whalen, 2001). The central nucleus of the amygdala (CNA) projects to brainstem structures involved in REM regulation and generation (Krettek and Price, 1978; Petrov et al., 1994; Takeuchi et al., 1982) and research has focused on its role in regulating sleep (Sanford et al., 2002; Sanford et al., 2006; Tang et al., 2005). However, involvement of other regions of the amygdala in the regulation of sleep is suggested by a report that bilateral electrolytic lesions of the basal amygdala (BA) increased NREM and REM in rats (Zhu et al., 1998) and that bilateral chemical lesions of the amygdala produced more consolidated sleep in chair restrained Rhesus monkeys (Benca et al., 1992). Electrical and chemical stimulation of BA also increase low voltage, high frequency activity in the cortical EEG and decrease NREM and total sleep time, respectively (Dringenberg and Vanderwolf, 1996; Zhu et al., 1998).

Strong expression of mGlu II receptors in BA (Gu et al., 2008; Tamaru et al., 2001) and the fact that BA regulates CNA output (Davis and Whalen, 2001) suggest that BA may be an important region for mediating the effects of mGlu II agents on sleep and arousal. There is also evidence from immunohistochemical (Gale et al., 2004; Hetzenauer et al., 2008; Petralia et al., 1996) and radioligand binding (Wright et al., 2001) studies of low to moderate expression of mGlu II receptors in the CNA. Therefore, in this study, we microinjected into BA and CNA various dosages of an mGlu II agonist (LY37) and antagonist (LY34) and determined their effects on sleep.

2. Results

2.1. Basal Nucleus of the Amygdala

2.1.1. Millimolar Dosage Range of LY37—We first examined sleep after microinjection into BA (n=6) of three millimolar (mM) concentrations of LY37 (3.2 mM (0.6µg/µl), 5.3mM (1.0µg/µl) and 10.7 mM (2.0µg/µl); 0.5µl) or vehicle alone (VEH;

distilled water). Figure 1 presents REM (Panel A) and NREM (Panel B) plotted as 2 h totals across the 20 h recording periods. For statistical analysis, the time spent in REM, NREM, total sleep and wakefulness were considered in 4h blocks over the 20h recording period. The resulting data were analyzed by one-way repeated measures ANOVAs for each of the five 4h blocks.

2.1.1.1. REM: The ANOVAs for REM amount found significant drug effects for the first [F(3, 15)=8.81, P<0.005], second [F(3, 15)=26.44, P<0.001] and third [F(3, 15)=6.17, P<0.01] 4h blocks. Compared to VEH, injection of LY37 at all three concentrations significantly decreased REM in the first, second and third 4h block (Table 1). There were no significant drug effects during the fourth or fifth 4h block.

The analyses for REM episode number found a significant drug effect for the first [F(3, 15)=11.972, P<0.001], second [F(3, 15)=19.79, P<0.001] and third [F(3, 15)=5.48, P<0.05] 4h block. The analyses were similar for REM episode duration with significant drug effect found for the first [F(3, 15)=9.60, P<0.001], second [F(3, 15)=7.17, P<0.005] and third [F(3, 15)=7.68, P<0.005] 4h block. Both REM episode number and REM episode duration were significantly decreased at all dosages during the first and second 4 h block and at the low and medium dosages during the third 4 h block (Table 1). There were no significant drug effects during the fourth or fifth 4h block.

2.1.1.2. NREM and total sleep: The ANOVAs for NREM amounts and total sleep did not reveal significant drug effects during any 4h block. The ANOVAs for NREM episode number and average NREM episode duration also did not find any significant drug effects.

2.1.2. Nanomolar Dosage Range of LY37 Because we did not observe a dosage dependent effect on REM or other sleep wake states after microinjections into BA of mM concentrations of LY37, we ran an additional experiment in which a separate group of rats (n=8) received microinjections of LY37 at the nanomolar level (0.1nM (1.8×10^{-8} µg/µl), 30nM (5.6×10^{-6} µg/µl) or 60nM (1.1×10^{-5} µg/µl); 0.5µl). Analyses were conducted as described above for the higher dosage range.

2.1.2.1 REM: During the first 4h block, the ANOVA for total REM (Figure 2A) found a significant drug effect [F(3, 21)=5.48, P<0.01]. Compared to VEH, injection of LY37 significantly decreased REM with injection at the high (P<0.01) and medium dosage (P<0.05). During the second 4h block, the ANOVA for REM found a significant drug effect [F(3, 21)=3.46, P<0.05]. Compared to VEH, injection of LY37 significantly decreased REM with injection at the high dosage (P<0.05). There were no significant drug effects during the other 4h blocks.

During the first 4h block, the ANOVA for REM episode number (Figure 2B) found a significant drug effect [F(3, 21)=5.09, P<0.01]. Compared to VEH, injection of LY37 significantly decreased REM episode number with injection at the high dosage. There were no significant drug effects during the other 4h blocks. During the first 4h block, the ANOVA for REM episode duration (Figure 2C) found a significant drug effect [F(3, 15)=9.20, P<0.001]. Compared to VEH, injection of LY37 significantly decreased REM episode number with injection at the high and medium dosages. There was also a significant difference between the low dosage and high dosage. During the second 4h block, the ANOVA for REM duration found a significant drug effect [F(3, 15)=5.41, P<0.01]. Compared to VEH, injection of LY37 significantly decreased REM with injection at the high dosage. There were no significant drug effects during any other 4h block.

2.1.2.2. NREM and total sleep: As with the higher range of concentrations, the ANOVAs for NREM amounts (Figure 3A), and total sleep (Figure 3B) did not reveal significant drug

effects. There also were no significant drug effects in the analyses for NREM average duration (Figure 3C) or episode numbers (Figure 3D).

2.1.3. Effects of LY34 on sleep—The data for REM, NREM, total sleep and Wakefulness after microinjection into BA of different dosages of LY34 (L: 1nM, M: 30nM, H:60nM) or vehicle (VEH; 0.01M PBS) alone were analyzed in 4 h blocks. The data analysis was conducted as described above and is presented in Table 2. The ANOVAs for total NREM found significant drug effects in the first [$F(3, 15)=6.43$, $P<0.01$] and second 4h block [$F(3, 15)=10.80$, $P<0.01$]. The high dosage of LY34 significantly decreased NREM compared to that of animals injected with vehicle or low and medium dosages of LY34. There were no other significant alterations in total NREM and there were no significant differences in the analyses for NREM episode number or average NREM duration in any of the 4h blocks.

The ANOVAs for total sleep found significant drug effects in the first [$F(3, 15)=7.47$, $P<0.01$], second 4h blocks [$F(3, 15)=9.89$, $P<0.01$]. The high dosage of LY34 significantly decreased total sleep compared to VEH and the low and dosages of LY34 in both blocks.

None of the REM parameters we examined were significantly altered at any of the dosages of LY34 that we tested.

2.2. Central Nucleus of the Amygdala

Microinjections of neither LY37 nor LY34 into CNA significantly altered any of the sleep parameters that we examined when the data were analyzed in 4h blocks (data not shown).

2.3. Microinjection Sites

Injection sites for BA are presented in Figure 4A. Although there was variation in placement, histology verified that each microinjection of LY37 or LY34 would have infused into the BA, though diffusion to other amygdaloid nuclei may also have been possible.

Injection sites in CNA are provided in Figure 4B. The histology for all rats microinjected with LY37 showed localizations within or immediately adjacent to CNA. Injection sites for microinjections with LY34 into CNA were found bilaterally in five of seven rats. In two rats, one of the cannula tracks terminated above CNA and the injection site could only be confirmed unilaterally. However, no differences in results were found in sleep whether these two rats were included or excluded from the analyses.

3. Discussion

Microinjection of the mGlu II agonist, LY37, into BA at both nM and mM concentrations significantly decreased REM without significantly altering NREM, total sleep or wakefulness. By comparison, microinjection of a high dosage of the specific mGlu II antagonist, LY34, into BA significantly suppressed NREM and total sleep thereby increasing wakefulness. These results demonstrate that activation of mGlu II receptors in BA significantly and specifically suppress REM in the rat whereas blocking mGlu II receptors can decrease sleep and enhance wakefulness. These data are consistent with the work of other researchers demonstrating that systemic administration of mGlu II agonists, LY37 and LY35, suppressed REM in a dosage-dependent manner (Ahnaou et al., 2009; Feinberg et al., 2002) whereas injection of LY34 increased arousal at the expense of sleep, though they noted decreases in REM as well as NREM (Feinberg et al., 2005). Further, these results show that BA plays a role in the regulation of REM and NREM as well as overall arousal level and suggest that it could be a site where mGlu II receptors exert their effects on

sleep. Specificity for BA as a site for metabotropic regulation of sleep is further suggested by findings that microinjections of LY37 and LY34 into CNA produced no significant alterations in sleep.

3.1. Effects of LY37 on sleep

The striking effect of microinjections of LY37 into BA was the pronounced and very selective decrease in REM over a wide range of concentrations. While the nM range of dosages produced a dosage response, all of the mM range of dosages we examined produced selective decreases in REM that persisted for 12 h or more post-injection (significant decreases were found during the first three 4 h blocks of the recording period). By comparison, a 1.0 mg/kg dosage of LY37 administered subcutaneously in the middle of the dark period totally suppressed REM for 6-h post-injection and reduced it by 80% over the next 6 h, but did not significantly alter NREM amounts (Feinberg et al., 2002). Thus, even though systemic administration of the drug would potentially impact widespread regions in the brain and administration during the dark period might be expected to produce greater reductions in REM, the effects on sleep were quite similar to those we observed with only localized administration into BA. This suggests that BA is a significant region for the regulatory effects of mGlu II receptors on sleep.

Agonists for mGlu II receptors may not alter basal glutamate release suggesting that other mechanisms may be responsible for the selective suppression of REM. This suggestion is consistent with the finding that systemic or local administration of LY37 reversed ketamine-evoked glutamate release in the medial prefrontal cortex but did not alter basal glutamate level (Lorrain et al., 2003). Moreover, systemic or local administration of LY37 significantly decreased GABA level in the hippocampus (Smolders et al., 2004) and mGlu receptor agonists suppress GABA release in various brain areas, (Anwyl, 1999; Cartmell and Schoepp, 2000). mGlu II receptors may also modulate the release of other neurotransmitters including acetylcholine and serotonin (Cartmell and Schoepp, 2000) which are implicated in the regulation of arousal and sleep. Therefore, it is possible that microinjection of LY37 into BA did not change basal glutamate release level but may have altered the release of other neurotransmitters which played roles in the alterations on REM.

3.2. Effects of LY34 on sleep

The injection of the antagonist, LY34, at the highest concentration (60nM) we tested significantly increased wakefulness at the expense of NREM and total sleep amounts, but did not significantly alter REM parameters. Lower concentrations of LY34 did not alter sleep and wakefulness compared to vehicle alone. Our results for local microinjections into BA are consistent with the presumed role of mGlu II receptors as inhibitory autoreceptors of glutamate release (Cartmell and Schoepp, 2000). The location of mGlu II receptors in the perisynaptic remote area in the presynaptic terminal suggests that these receptors may only work under conditions of high glutamate release; that is, released glutamate has to reach a level sufficient to travel to the sites of mGlu II receptors that are far away from the release site (Cartmell and Schoepp, 2000; Schoepp, 2001). For example, under repetitive stimulation, hippocampal mossy fiber synapses increase glutamate release and glutamate concentration that allows glutamate to spread away from the synapse and activate mGlu II receptors (Scanziani et al., 1997). The activated mGlu II receptors subsequently suppressed glutamate release. Thus, blockade of these receptors with an antagonist could contribute to a shift to higher glutamate release thereby increasing the activity level in BA. This increase of activity in BA could underlie the increase in arousal level and decrease in sleep.

Systemic administration of either LY37 or LY34 in the dark period suppressed REM although co-administration of LY34 attenuated the reduction of REM produced by LY37

(Feinberg et al., 2002; Feinberg et al., 2005). These authors explained the seemingly contradictory effects of LY37 and LY34 based on a “one-stimulus” model of NREM-REM alternation (Feinberg and March, 1988; Feinberg and March, 1995). In this model, REM sleep is an intermediate state between the high arousal of wakefulness and the low arousal of NREM. Thus, REM could be decreased both by drugs that increase neural excitation and arousal and by drugs that decrease neural excitation and arousal by converting REM to wakefulness or NREM, respectively. This model may help to explain the reduction in REM produced by systemic administration of LY34 at a phenomenological level. Systemic administration of LY34 has also been shown to increase anxiety-related behavior in the elevated plus maze (Linden et al., 2005) suggesting that enhanced arousal associated with altered emotional state could have been a factor in the reductions in sleep. The decrease with systemic LY34 may also be enacted at a site outside BA. Of course, microinjection into BA of a different concentration of LY34 might have significantly altered REM. This possibility is suggested by the apparent (though non-significant) decreases seen with at 30 and 60 nM doses of LY34.

3.3. BA as a regulator of REM

A few previous studies have implicated the BA in the general regulation of sleep (e.g., (Dringenberg and Vanderwolf, 1996; Zhu et al., 1998)); however, the CNA has been most implicated in the regulation of REM (Calvo et al., 1996; Sanford et al., 2002; Sanford et al., 2006). CNA has direct projections to brainstem areas involved in the generation and regulation of REM (e.g., locus coeruleus, laterodorsal tegmental nucleus and dorsal raphe nucleus (Krettek and Price, 1978; Petrov et al., 1994; Takeuchi et al., 1982)) that provide a substrate for amygdalar regulation of REM. Most functional data are consistent with the hypothesis that inactivation of CNA inhibits REM. For example, inactivation of the CNA with muscimol, a GABA_A agonist, selectively decreased REM without altering NREM whereas microinjection of GABA_A antagonist bicuculline into CNA increased REM (Sanford et al., 2002). Temporary inactivation of CNA by tetrodotoxin, a sodium channel blocker, also significantly suppressed REM and reduced arousal as indicated by shortened NREM latency and decreased activity in an arousing environment (Tang et al., 2005). While we cannot exclude the possibility that LY37 or LY34 diffused into the CNA, the putative modes of action (as discussed above, LY37 may reduce GABA release which in CNA should increase REM and LY34 may enhance glutamate release which in CNA should enhance REM) suggest that this was unlikely. However, BA projects to CNA and regulates its output as part of a “central fear system” (Davis and Whalen, 2001). BA therefore likely controls CNA influences on REM which is significantly reduced by conditioned fear (Jha et al., 2005a; Sanford et al., 2003a; Sanford et al., 2003b; Sanford et al., 2003c). BA also projects to the bed nucleus of the stria terminalis (BNST) which has projections to many REM-related brainstem regions similar to those of CNA (Davis and Whalen, 2001), thereby providing an additional pathway by which BA could influence REM.

Two types of neurons are found in BA. The first type is pyramidal neurons which comprises the majority of the total neuronal population. These neurons demonstrate a high level of immunoreactivity for glutamate and are considered glutamatergic neurons (Smith and Pare, 1994). The second type of are smaller, local circuit GABAergic interneurons (Pitkanen and Amaral, 1994) that send inhibitory projections to the pyramidal neurons, which, in turn, send excitatory projections to intercalated cells (Pitkanen, 2000; Sah et al., 2003) that are small GABAergic cell bodies located between BA and CNA (Millhouse, 1986). The excitatory inputs from the BA would thus generate inhibitory effects on neurons of CNA (Royer et al., 1999). Based on this circuitry, infusion of LY37 into BA could suppress the local release of GABA that would allow increased excitatory inputs to intercalated neurons. The end result would be an increase in inhibitory signals to neurons to CNA, thereby suppressing CNA

activity and decreasing REM. This suggested mechanism is consistent with studies that found suppressing activity in CNA decreased REM (Sanford et al., 2002; Sanford et al., 2006). It is also consistent with *in vitro* findings that the application of LY35 and LY37 can inhibit projection neurons in the basal and lateral amygdala via direct hyperpolarization or by blockage of excitatory input (Muly et al., 2007).

3.4. Conclusions

The current study demonstrates that BA likely plays a major role in the effects of mGlu II agents in the regulation of sleep. mGlu II receptors also have been reported to be involved in stress and anxiety (Palucha and Pilc, 2007) and mGlu II agonists have been found to have anxiolytic effects in several animal models (Helton et al., 1998). For example, oral administration of the agonist, LY35 in mice increased open-arm activity in the elevated plus maze (Monn et al., 1997). In contrast, the antagonist, LY34 appears to have anxiogenic properties as intraperitoneal injections of LY34 increased plasma corticosterone in mice (Scaccianoce et al., 2003) (note though that other authors have reported anxiolytic effects for mGlu II antagonists (Shimazaki et al., 2004; Yoshimizu et al., 2006)). The amygdala has a central role in anxiety and fear and BA is involved in both the acquisition and expression of conditioned fear (Gale et al., 2004; Ono et al., 1995; Yaniv and Richter-Levin, 2000) as well as in the extinction of fear (Myers and Davis, 2007; Quirk and Mueller, 2008) and it is likely involved in the anti-anxiety effects of mGlu II agonists. Sleep disturbances are prominent symptoms of anxiety and stress-related disorders which likely also involve the amygdala (Liu et al., 2009). Thus, work is needed to determine whether mGlu II agents can modulate alterations in sleep associated with stress and anxiety.

4. Experimental Procedure

4.1. Subjects

The subjects were male Wistar rats obtained from Harlan (Indianapolis, IN). The rats were approximately 300 gm at the time of surgery. The rats were individually housed in polycarbonate cages and given ad lib access to food and water. The room for sleep recording/housing was kept on a 12:12 light:dark cycle with lights on from 7:00 AM to 19:00 PM eastern standard time. Room temperature was maintained at $24.5^{\circ} \pm 0.5^{\circ}\text{C}$.

4.2. Surgery

One week following their arrival, the rats were implanted with skull screw electrodes for recording the electroencephalogram (EEG) and with stainless steel wire electrodes sutured into the nuchal muscles for recording the electromyogram (EMG). Leads from the implanted electrodes were routed to a 9-pin miniature plug attached to the skull. Guide cannulae (26 gauge) for microinjections were bilaterally implanted to introduce the tip of the injection cannulae into the BA (A 6.4, ML ± 4.8 , DV 8.0) or CNA (A 6.8, ML ± 4.0 , DV 7.5). All surgeries were conducted under aseptic conditions. The rats were anesthetized with isoflurane (5% induction; 2% maintenance). Ibuprofen (15mg/kg) was available in their drinking water 24h before surgery and for 3 days after surgery to alleviate pain. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Experimental Animals (Protocols #07-005 and #09-019).

4.3. Drug preparation

Both the agonist (LY37) and antagonist (LY34) were purchased from Tocris Bioscience (Ellisville, MO). Immediately prior to the microinjection, LY37 was dissolved in distilled water which served as vehicle. LY34 was dissolved in 0.01 M phosphate buffered saline (PBS) which served as vehicle.

4.4. Microinjection Procedures

After surgery, the rats were allowed 14 days for recovery. Then they were habituated to microinjection procedures for two days. This included handling and removing and replacing the dummy cannulae as required for an actual microinjection. After each habituation handling session, sleep was recorded for 20h.

For the drug studies, six rats received bilateral microinjections of LY37 into the BA at three millimolar concentrations (3.2 mM (0.6 μ g/ μ l), 5.3mM (1.0 μ g/ μ l) or 10.7 mM (2.0 μ g/ μ l)) and vehicle alone. Eight rats received bilateral nanomolar microinjections of LY37 into the BA and 8 rats received bilateral nanomolar microinjections of LY37 into the CNA at three concentrations (0.1nM (1.8 $\times 10^{-8}$ μ g/ μ l), 30nM (5.6 $\times 10^{-6}$ μ g/ μ l) or 60nM (1.1 $\times 10^{-5}$ μ g/ μ l)) and vehicle alone. Six rats received bilateral microinjections into the BA and 7 rats received bilateral microinjections into the CNA of three concentrations of the antagonist, LY34 (1nM, 30nM or 60nM) and vehicle alone. The volume for microinjections into BA was 0.5 μ l and the volume for microinjections into CNA was 0.2 μ l.

For microinjections, cannulae (33 ga.), which projected 1.0 mm beyond the tip of the guide cannulae, were secured in place within the guide cannulae. The injection cannulae were connected to lengths of polyethylene tubing that in turn were connected to 5.0 μ l Hamilton syringes. The injection cannulae and tubing had been pre-filled with the solution to be injected. Solutions in a volume of 0.5 μ l were slowly infused over 3 min, with the cannulae in place 1 min prior to the start and 1 min after the completion of the microinjection.

For each drug, microinjections were administered in a counterbalanced order at 5-day intervals. Following each microinjection, the rats were returned to their home cages and connected to recording cables. Sleep was then recorded for 20 hours.

4.5. Sleep recording

Sleep recording was performed with the rats in their home cages. A lightweight, shielded cable was connected to a plug in the rats' head. The cable was connected to a swivel that allowed free movement for the rats within their cages. EEG and EMG signals were processed by a Grass Model 12 polygraph equipped with model 12A5 amplifier and routed to an A/D board (Eagle PC30) housed in a Pentium class PC. The signal were digitized at 128 HZ and collected in 10-second epochs using a custom sleep data collection program.

4.6. Determination of sleep

The EEG and EMG records were visually scored by trained observer to determine wakefulness, NREM and REM sleep in 10-second epochs (Sanford et al., 2002). Wakefulness was determined by the presence of low-voltage, fast EEG; high-amplitude, tonic EMG levels; and phasic EMG bursts that could be associated with gross body movements. NREM was scored by the presence of spindles interspersed with slow waves, lower muscle tone, and no gross body movements. REM was determined by the presence of low-voltage, fast EEG, theta rhythm, and muscle atonia.

4.7. Histology

The rats were anesthetized with isoflurane (inhalation: 5% induction, 2% maintenance) and then transcardially perfused intracardially with 0.9% SAL and 10% formalin. The brains were processed to determine cannula placements. For this purpose 40 μ m slices were made through the areas of interest with a cryostat, and the sections were stained with cresyl violet. Injection sites in BA and CNA were verified by comparing sections to those in the stereotaxic atlas (Paxinos and Watson, 1998).

4.8. Data analysis

Statistical analyses were conducted using SigmaStat (SPSS, Inc., Chicago, Illinois). One-way repeated measures ANOVAs across drug treatment were used to analyze potential changes in sleep in 4h blocks. The Tukey test was used to make comparisons among means when warranted.

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Highlights

- We tested the effects of Group II metabotropic (mGlu II) agents in the amygdala on sleep.
- LY379268, mGlu II agonist, injected into basal amygdala decreased rapid eye movement sleep.
- LY341495, mGlu II antagonist, injected into basal amygdala decreased sleep.
- Neither drug injected into central nucleus of the amygdala impacted sleep.
- The basal amygdala is a site of action for the effects of mGlu II agents on sleep.

Figure 1.

Selected sleep parameters (mean $\pm$ SEM) plotted for 2h periods for rats that received intra-amygdala microinjections of the millimolar range of LY37 (L: 3.2 mM; M: 5.3mM; H: 10.7 mM): REM (A), NREM (B), REM episode number (C) and average REM episode duration (D). VEH: vehicle control; L: 3.2mM; M: 5.3mM; H: 10.7mM. The dark bar on the X-axis indicates the dark period.

Figure 2.

Plots of total REM amounts (A), REM average duration (B) and REM episode number (C) in 4h blocks (mean $\pm$ SEM) after microinjection into the amygdala of the nanomolar range of LY37 (L: 0.1nM, M: 2nM or H: 10nM) or VEH (vehicle). The dark bar on the X-axis indicates the dark period. *, $P < 0.05$, significant difference relative to VEH; **, $P < 0.05$, significant difference between drug conditions. Comparisons were conducted using Tukey tests.

Figure 3.

Plots of total NREM amounts (A), total sleep (B), NREM average duration (B) and NREM episode number (C) in 4h blocks (mean±SEM) after microinjection into the amygdala of the nanomolar range of LY37 (L: 0.1nM, M: 2nM or H: 10nM) or VEH (vehicle). The dark bar on the X-axis indicates the dark period. There were no significance differences across treatments for any of the measures.

Figure 4. Line drawings showing placements of microinjection cannulae in the (A) basal amygdala (BA) and (B) central nucleus of the amygdala (CNA). Dark triangles indicate injection sites for millimolar concentrations of LY37. Dark squares indicate injection sites for nanomolar microinjections of LY37. Gray circles indicate injection sites for the antagonist.

Table 1

Time spent (min) in REM, REM episode number and REM average duration and total NREM and total sleep time (mean $\pm$ SEM) during 4h blocks (b1–b5) after microinjection of vehicle (VEH, distilled water) and LY37 (low 3.2 mM (0.6 μ g/ μ l), medium 5.3mM (1.0 μ g/ μ l) or high 10.7 mM (2.0 μ g/ μ l); 0.5 μ l) dosages into BA.

	Vehicle	Low	Median	High
REM Amount				
b1	13.5 (2.6)	3.8 (1.2)*	3.0 (1.2)*	3.3 (0.9)*
b2	16.8 (1.2)	4.6 (1.4)*	3.3 (1.1)*	3.5 (1.4)*
b3	14.0 (1.9)	7.2 (2.5)*	4.7 (2.8)*	5.7 (2.4)*
b4	10.9 (1.6)	8.4 (2.3)	5.8 (1.8)	7.8 (2.3)
b5	6.3 (2.1)	4.4 (0.8)	3.1 (0.4)	1.8 (0.5)
REM Episode Number				
b1	7.7 (1.3)	2.2 (0.6)*	1.0 (0.5)*	2.8 (1.3)*
b2	10.3 (1.3)	1.5 (0.5)*	2.8 (1.1)*	3.3 (1.0)*
b3	11.7 (1.7)	4.2 (1.8)*	3.3 (2.7)*	5.8 (1.9)
b4	9.7 (1.3)	9.3 (2.4)	5.3 (2.2)	7.7 (1.7)
b5	5.8 (2.0)	1.7 (0.5)	2.6 (0.8)	4.5 (1.1)
REM Average Duration				
b1	91.8 (14.7)	23.8 (8.9)*	16.7 (7.9)*	25.4 (13.2)*
b2	108.5 (15.6)	28.3 (10.8)*	29.2 (11.1)*	44.2 (13.7)*
b3	79.1 (6.8)	39.6 (15.0)*	17.2 (9.3)*	53.7 (13.5)
b4	60.3 (10.6)	36.5 (9.2)	33.9 (12.3)	55.1 (7.7)
b5	28.4 (7.1)	17.4 (7.1)	26.7 (7.3)	26.4 (7.2)
NREM Amount				
b1	140.3 (10.4)	156.2 (11.2)	118.3 (21.1)	134.0 (7.7)
b2	112.3 (9.7)	125.3 (13.3)	126.1(10.3)	135.0 (12.9)
b3	54.6 (1.9)	71.3 (11.3)	79.2 (14.0)	73.8 (17.3)
b4	58.9 (10.9)	84.5 (6.9)	88.1 (16.1)	90.7 (10.4)
b5	50.5 (8.9)	57.2 (8.2)	72.1 (13.9)	79.8 (12.3)
Total Sleep				
b1	153.8(11.9)	159.4 (11.7)	121.3 (22.1)	137.7 (8.4)
b2	129.2 (10.7)	128.8 (13.6)	129.4 (10.2)	139.6 (12.2)
b3	68.6 (3.2)	77.0 (12.0)	84.0 (13.0)	81.0 (16.6)
b4	69.9 (12.3)	92.3 (7.5)	93.8 (16.1)	99.1 (9.1)
b5	56.8 (10.9)	58.9 (8.0)	75.2(13.7)	84.2 (12.1)

* P<0.05, significant difference relative to vehicle. Comparisons were conducted with Tukey tests.

Table 2

Amounts of REM and NREM, and total sleep time (mean $\pm$ SEM) during 4h blocks (b1–b5) after microinjection of vehicle (PBS) or LY34 (low: 1.0 nM, medium: 30 nM and high: 60 nM) into the basal amygdala.

	Vehicle	Low	Median	High
REM Amount				
b1	9.4 (2.5)	10.8 (1.6)	7.4 (1.8)	8.0 (1.9)
b2	13.7 (2.6)	13.0 (3.6)	11.3 (3.4)	10.2 (3.2)
b3	12.6 (3.2)	14.7 (4.7)	14.5 (6.5)	9.7 (4.3)
b4	12.8 (5.5)	14.7 (4.8)	13.2 (5.6)	12.2 (5.0)
b5	6.7 (2.2)	9.4 (2.5)	5.8 (1.9)	8.2 (2.8)
NREM Amount				
b1	114.8 (8.2)	113.9 (5.3)	115.7 (9.5)	97.0 (6.5)*
b2	90.6 (4.0)	93.4 (5.1)	88.8 (6.6)	73.5 (4.9)*
b3	45.9 (5.5)	48.8 (7.4)	56.2 (9.1)	42.9 (5.6)
b4	54.1 (9.4)	57.3 (8.0)	58.5 (6.7)	50.3 (5.8)
b5	60.0 (12.4)	75.2 (11.0)	69.9 (11.5)	67.6 (14.5)
Total Sleep				
b1	124.2 (6.6)	124.6 (6.4)	123.2 (8.3)	105.0 (8.1)*
b2	104.3 (5.9)	106.4 (7.5)	100.1 (9.1)	83.7 (6.7)*
b3	58.5 (8.6)	63.5 (11.6)	70.6 (14.94)	52.6 (9.4)
b4	66.9 (14.1)	72.0 (12.2)	62.6 (10.4)	71.7 (12.0)
b5	66.6 (13.5)	84.6 (12.7)	75.6 (12.9)	75.0 (16.6)

* $P < 0.05$, significant difference relative to vehicle and to each of the lower dosages of LY34. Comparisons were conducted using Tukey tests.