INACTIVATION OF NEURONAL FUNCTION IN THE AMYGDALOID REGION REDUCES TAIL ARTERY BLOOD FLOW ALERTING RESPONSES IN CONSCIOUS RATS

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Abstract

Few studies have investigated whether neuronal function in the amygdaloid complex is necessary for the occurrence of the cardiovascular response to natural (unconditioned) environmental threats. In the present investigation in conscious unrestrained Sprague-Dawley rats we inactivated neuronal function in the amygdaloid complex acutely (bilateral muscimol injections) or chronically (unilateral or bilateral ibotenic acid injections) and measured the effect on sudden falls in tail artery blood flow elicited by non-noxious salient stimuli (sympathetic cutaneous vasomotor alerting responses, SCVARs). After acute bilateral injection of vehicle (200nl Ringer’s solution) the SCVAR index was 81±2%, indicating that tail blood flow was reduced by 81% in response to the salient stimuli. After acute bilateral injection of muscimol (1nmol in 200nl of Ringer’s solution) into the amygdaloid complex the SCVAR index was 49±5%, indicating that tail blood flow was reduced by 49% in response to the salient stimuli (P<0.01 vs vehicle, n=7 rats for vehicle and 6 for muscimol). One week after unilateral ibotenic acid lesions, the SCVAR index was 68±3%, significantly less than 90±1%, the corresponding value after unilateral injection of vehicle (p<0.01, n=6 rats in each group). After bilateral ibotenic acid lesions the SCVAR index was 52±4%, significantly less than 93±1%, the corresponding value after bilateral injection of vehicle (p<0.001, n=6 rats in each group). Ibotenic acid caused extensive neuronal destruction of the whole amygdaloid complex, as well as lateral temporal lobe structures including the piriform cortex. Our results demonstrate that the amygdaloid complex plays an important role in mediating the tail artery vasoconstriction that occurs in rats in response to the animal’s perception of a salient stimulus, redirecting blood to areas of the body with more immediate metabolic requirements.
Keywords
amygdala
cutaneous blood flow
vasoconstriction
stress
ibotenic acid
muscimol
INTRODUCTION

Relatively few studies have investigated the role of the amygdaloid complex in behavioral and autonomic responses to naturally occurring environmental events. Blanchard and Blanchard studied behavioral changes in rats with bilateral radiofrequency lesions of the amygdaloid complex (Blanchard and Blanchard, 1972; Kemble et al., 1984; Kemble et al., 1990). The lesioned rats frequently approached a cat (sedated) introduced into the cage, and even climbed onto the cat. Blanchard and Blanchard concluded that the amygdala is involved in regulating the emotional-motivational state associated with defensive behaviors. Werka et al. (1978) showed that rats with lesions of the central nucleus of the amygdala readily cross an open field, rather than remain close to the wall. Fox and Sorenson (1994) also implicated the amygdala in natural fear responses, but contrasting findings have also been reported (Sananes and Campbell, 1989; Watkins et al., 1993). Generally similar behavioral changes were also observed in primates after amygdaloid lesions (Kluver and Bucy, 1939; Weiskrantz, 1956).

The studies cited above did not assess effects on autonomic cardiovascular variables. The amygdaloid complex has been shown to be part of the brain neural circuitry mediating cardiovascular responses to Pavlovian classically conditioned fear stimuli (LeDoux et al., 1986; LeDoux et al., 1988; LeDoux et al., 1990; LeDoux, 2000; Davis and Whalen, 2001; Gozzi et al., 2010; Pape, 2010; Pape and Pare, 2010). However only a few studies have documented the effect of amygdaloid lesions on autonomic cardiovascular responses to unconditioned stimuli (Galeno et al., 1984; Kubo et al., 2004; Fortaleza et al., 2009). Thus the role of amygdaloid nuclei in
mediating cardiovascular responses to natural environmental threats is still largely undefined.

Thermoregulatory cutaneous vascular beds (ear pinna in rabbits and tail artery in rats) are extremely sensitive to the detection of significant, potentially threatening, environmental events, events that are salient for the life of the animal (Yu and Blessing, 1997b; de Menezes et al., 2009). When an animal detects such an event, the sympathetic outflow to the cutaneous beds is activated, causing a robust and substantial reduction in blood flow. In both rabbits and rats, these sudden falls in cutaneous blood flow are preceded by a sudden increase in the proportion of theta (5-8 Hz) rhythm in the hippocampal EEG, documenting that the animal has detected the salient environmental event (Yu and Blessing, 1997b; de Menezes et al., 2009). We refer to the sudden reductions in cutaneous blood flow as SCVARs (sympathetic cutaneous vasomotor alerting responses) (Blessing, 2005; de Menezes et al., 2009). The associated increases in arterial blood pressure are quite modest, and the changes in heart rate vary with the nature of the stimulus (Galeno et al., 1984; Yu and Blessing, 1997b). Diversion of blood from the cutaneous bed may reduce the risk of hemorrhage in case of actual physical attack, and the blood can be redirected to areas of the body with more urgent metabolic requirements, including the brain (Yu and Blessing, 1997a; Blessing, 2003).

In rabbits, functional inactivation of the amygdala by focal microinjections of either tetrodotoxin or the long acting GABA-A receptor agonist muscimol, substantially reduces SCVARs (Yu and Blessing, 1999, 2001). The present investigation was conducted to determine whether inactivation of the amygdaloid complex also reduces SCVARs in rats. We measured the tail artery blood flow response to salient stimuli in conscious rats after bilateral injection of muscimol into
the amygdaloid complex, or after prior bilateral lesioning of neurons in the amygdaloid complex by focal microinjections of ibotenic acid.

**EXPERIMENTAL PROCEDURES**

*Animals and surgical procedures*

Experiments, approved by the Animal Welfare Ethical Committee of Flinders University, were carried out on 37 male Sprague-Dawley rats (300-400 g). Care was taken to minimize the number of animals. For implantation of measuring devices rats were anaesthetized with 2% isoflurane (Veterinary Companies of Australia Pty, Ltd., NSW, Australia) in 100% oxygen. An ultrasonic Doppler blood flow probe (Iowa Doppler Products, IA, USA) was implanted around the tail artery about 2 cm distal to the base (Garcia et al., 2001; Ootsuka et al., 2009). After cannula implantation for amygdala injections (see below) flow probes were connected via subcutaneous wires to a head-piece attached to the skull.

*Preparation for acute injection of muscinol into the amygdaloid complex*

Anesthetized rats were placed in a stereotaxic apparatus (Stoelting Stereotaxic Instruments, USA) and via burr hole access, stainless steel guide cannula (26 gauge, Plastics One, Roanake, VA) were positioned bilaterally, 1 mm dorsal to the central nucleus in the amygdaloid complex (AP -2.3 mm; ML +4.8 mm; DV 7.0 mm, (Paxinos and Watson, 1986)). They were held in place with stainless steel skull screws fixed to the skull and quick drying dental cement. Stainless steel stylets (33 gauge), with plastic caps, were inserted into the guide cannulae. The head socket for the tail probe wires was fixed to the skull using dental cement. All rats were treated post-operatively with analgesic (Caprofen, 5 mg/kg s.c, Norbrook Laboratories, Melbourne) and antibiotic (Baytril, 15 mg/kg s.c, Bayer Australia, Sydney). Animals recovered in the Animal House for at least 1 week.
On the day of the experiment the rat was transferred to a wooden box (40 x 40 x 40 cm, temperature 24-26°C) with a swivel device that could be connected by a flexible cable to the head socket. The tail artery Doppler blood flow signal was continually recorded for 30 min (see below) with the animal left undisturbed. The stylet was then removed from the guide cannula and replaced with an injection cannula (Hypotube S/S 304-RW stainless steel 33 gauge, Small Parts, Inc. Miramar, FL, USA), with the ventral tip protruding 1 mm from the tip of the guide cannula. The proximal end of the injection cannula was already attached to plastic tubing (Polypropylene Dural Plastics & Engineering, Auburn, NSW, Australia) connected to a calibrated 5 µl glass micropipette (Clay Adams, Division of Becton, Dickinson and Company, Parsippany, NJ). Either muscimol (Sigma, St Louis) or vehicle (ringer) was pressure injected using an air-filled syringe attached to the micropipette. Movement of a fluid-air meniscus in the micropipette was used to monitor the volume of the injection. Muscimol (1nmol in 200 nl of ringer) or vehicle was injected bilaterally over a period of approximately 1 min. The injection cannula was removed after an additional 1 min, and replaced by the stylet.

The rat was then left undisturbed for 20 min, and then a series of 6 standardized stimuli were administered (see below) and the effect on tail artery blood flow was recorded. The animal was then returned to the Animal House. Each rat received no more than 2 bilateral injections, one of muscimol and one of vehicle in counterbalanced order, with at least 2 days between injections. The injection sites for the first injection were marked by including fluorescent beads (FluoSpheres, Molecular Probes, Oregon) in the injectate. The injection sites for the second injection was marked by including a crystal of Horseradish Peroxidase (Sigma V1, St Louis) dissolved in the injectate.
Rats were anesthetized and placed in the stereotaxic apparatus. Unilateral or bilateral burr holes were made in the skull. A small cut was made in the dura mater. A long-shanked 5 μl glass micropipette (Accu-Fill 90, Micropet, Clay Adams, NJ), calibrated in 100 nl steps, was filled with ibotenic acid solution (Tocris Bioscience, Bristol) or vehicle and the position of a fluid-air meniscus in the micropipette was monitored. The tip of the micropipette was lowered into the basolateral amygdala (AP -2.3 mm; ML +4.8 mm; DV 8.0 mm) (Paxinos and Watson, 1986). Ibotenic acid (5 μg in 250 nl) or vehicle was injected during approximately 1 min, and the pipette left in place for an additional 1 min. The cannula was then withdrawn. Further surgery for implantation of a Doppler flow probe around the base of the tail artery and a head-piece attached to the skull, as described above. Animals were returned to the Animal House for at least one week before experimentation.

On the day of the experiment, lesioned or control rats were transferred to the wooden box in the experimental room (as for muscimol experiments described above). The tail artery Doppler blood flow signal was continually recorded for 1 hour with the animal left undisturbed. The standardized stimuli (see below) were then administered and the effect on tail artery blood flow recorded. Stimuli were administered at times when the tail artery blood flow was at a high level.

Salient stimuli protocol

In previous studies (Yu and Blessing, 1997b; de Menezes et al., 2009) we have demonstrated that environmental events that attract the animal’s attention, salient events as defined by a sudden increase in the power of hippocampal theta rhythm, also lead to a sudden fall in cutaneous blood flow. We have standardized a series of 6 stimuli, delivered in a constant order with at least 5 min between stimuli. These
stimuli were used in the acute muscimol and chronic ibotenic acid experiments. 1) A flexible metal rod was released from a restraint so that it suddenly tapped the side of the wooden box. 2) A 0.5 s, 90 dB, 100 Hz sound was made outside the box. 3) The box was dropped 1.5 cm by sudden removal of a support under the box. 4) The box was vigorously moved to and fro 2-3 times. 5) A small window (15x15 cm) in the front of the box was suddenly opened. 6) The door of the box was opened and a single pinprick over the thigh was administered using a 23 G sterile needle.

Data recording and statistical analysis

The tail artery Doppler signal was transmitted via the subcutaneous wires to the head piece and then, via a flexible cable and a swivel device to a System 6 Model 200 (Triton Technology, San Diego) device that converted a frequency difference to a voltage. The voltage signal was calibrated in cm/s using the Triton internal calibrator. The voltage signal was then transferred to a MacLab/s device programmed with Chart 7 software (ADinstruments Inc, Castle Hill) for signal sampling (40 Hz) and analogue to digital conversion. The piezo-electric vibration sensor was also connected to the MacLab and sampled at 10 Hz. Chart files were then exported to a Macintosh computer programmed with Chart and IgorPro software (Wavemetrics, Lake Oswego).

In the acute muscimol or vehicle experiments the mean and the coefficient of variation (CV, expressed as a percentage) of the tail artery Doppler flow signal were calculated for the 20 min control pre-injection period, and for the 20 min post-injection period (commencing 5 min after the injection). In the chronic ibotenic acid or vehicle experiments similar parameters were calculated for the 20 min undisturbed period before the administration of the first experimental stimulus (tap on the cage). In both the acute and chronic experimental models we quantified the change in the tail
artery Doppler flow signal using the SCVAR index. We selected a 3s signal sample just before administration of each of the 6 salient stimuli, and a post-stimulus signal sample (3s sample at lowest flow level in the 10 s after the stimulus onset). The SCVAR index calculation uses both mean blood flow and the average amplitude of each individual tail artery pulse during the 3s samples. The SCVAR index formula is $100 - \frac{(\text{post-stimulus mean flow} + \text{post-stimulus mean pulse amplitude})}{(\text{pre-stimulus mean flow} + \text{pre-stimulus mean pulse amplitude})} \times 100$, so that a large fall in the Doppler flow signal leads to a high SCVAR index. To simplify the presentation of results, in each experimental condition the separate indices for the 6 stimuli were averaged to provide a single SCVAR index value for each rat in each condition.

Group data are shown as mean±sem unless otherwise indicated. Group results were analysed statistically using Statview (SAS institute, Carey, NC, USA) software. In the acute muscimol or vehicle injection experiments pre- and post- injection mean flow, CV and averaged SCVAR index values were compared using repeated measures ANOVA. In the chronic model, effects of ibotenic acid versus vehicle were compared using factorial ANOVA. In addition, for SCVAR index values for each individual stimulus, we compared muscimol versus vehicle and bilateral ibotenic acid versus vehicle, using factorial ANOVA. Fischer’s protected least significance difference test was used to determine significant post hoc differences between groups, with the primary analysis significance threshold set at 0.05 level.

**Histological examination of injection and lesion sites**

After completion of experiments using ibotenic acid injections, rats were anesthetized with pentobarbital (100 mg/kg i.p.) and brains were perfused transcardially with aldehyde fixatives, removed and left in the fixative with 30% sucrose. Serial sections (50 µm) were cut from the forebrain using a freezing
microtome (Leitz) and stained for Nissl substance using Neutral Red. After muscimol treatment, the sections were processed for HRP reaction product, visualized with the Diaminobenzidine (DAB, Sigma, St Louis) reaction. The fluorescent beads were examined in an AX50 fluorescence microscope (Olympus).

RESULTS

Bilateral muscimol injections into the amygdaloid complex

Figure 1 shows tail artery blood flow signals before and after bilateral injection of vehicle or muscimol into the amygdaloid complex. In the pre-injection signals, for both muscimol and vehicle injected rats, the tail artery blood flow signal shows frequent apparently spontaneous sudden falls to near zero levels, with gradual recovery to a high flow level. Muscimol injections substantially reduced these sudden falls, as is apparent in the group data for coefficient of variation (Table 1). Muscimol also increased the mean value of the tail flow signal (Table 1), principally because it substantially reduced the frequency of the sudden falls in flow. The combined stimuli SCVAR index results in Table 1 document that muscimol, but not vehicle, also substantially reduced the falls in tail artery blood flow elicited by experimental administration of the stimuli (Table 1). Figure 4A shows that muscimol reduced the SCVAR index for each of the 6 individual stimuli, in comparison with vehicle-injected animals.

Ibotenic acid injections into the amygdaloid complex

Records from individual animals demonstrating the resting tail blood flow Doppler signal at least one week after bilateral ibotenic acid or vehicle injection are shown in Fig. 2 and expanded records of the SCVAR response to a tap stimulus after vehicle, unilateral or bilateral injections of ibotenic acid are shown in Fig. 3. Group results for unilateral and bilateral injections are shown in Table 2. The coefficient of
variation of the tail flow signal and the overall SCVAR index (results for individual stimuli combined) were significantly reduced in comparison with vehicle-injected animals. The combined SCVAR index was lower after bilateral compared with unilateral injections. Figure 4B shows that bilateral injections of ibotenic acid substantially reduced the SCVAR responses to each of the 6 individual stimuli in comparison with vehicle-injected animals. After bilateral injections of ibotenic acid the SCVAR index was reduced to a value not significantly different from the corresponding value after bilateral injection of muscimol (Table 2).

Histology

Figure 5 represents the histology of coronal sections of rat brains. Fig. 5A, shows the bilateral guide cannulae tracts and the site of injections of muscimol in the amygdaloid complex. The DAB reaction showing the spread of HRP reaction product occurred at the site of injection. Fluorescent beads were also injected to trace out the injections sites (fig. 5B). Brain sections with ibotenic acid lesions were treated with neutral red for Nissl staining. Figure 5C, shows the effect of vehicle injection in the amygdaloid complex; in contrast figure 5D, represents the effect of ibotenic acid which clearly shows the substantial reduction in the visible cell nuclei in the whole amygdaloid complex. The spread of toxin also lesioned the lateral portion of temporal lobe structures, including the piriform cortex.

DISCUSSION

Cutaneous blood flow as an index of the perception of salient events

Our previous publications have documented that sympathetic control of thermoregulatory cutaneous blood flow is exquisitely responsive to the perception of salient environmental events (Yu and Blessing, 1997b; de Menezes et al., 2009). In
the conscious rabbit (ear pinna blood flow) or rat (tail blood flow) cutaneous blood flow may suddenly fall to near zero levels either spontaneously or in response to apparently minor events, for example when the experimenter enters the room or when the laboratory telephone rings (Yu and Blessing, 1997b). Thus the coefficient of variation of cutaneous blood flow is substantially greater than the variation of other cardiovascular parameters (including heart rate and arterial pressure), even in the absence of experimentally administered stimuli (Yu and Blessing, 1997b; Yu and Blessing, 1999, 2001). The substantial response of the cutaneous vascular bed to salient stimuli contrasts with relatively minor changes in arterial pressure and in blood flow to other vascular beds (Yu and Blessing, 1997b). Even when the experimentally introduced stimulus is probably in the stressful range, for example a 110 db noise, changes in blood flow to non-cutaneous vascular beds are minor (Galeno et al., 1984; de Menezes et al., 2009).

Sudden falls in tail artery blood flow in rats, quantified by the SCVAR index, provide a sensitive measure of the animal’s perception of salient environmental events. It is not essential for the event to be noxious or obviously stressful. Diversion of blood from the cutaneous beds could reduce the risk of hemorrhage in the case of actual physical attack, and increase the proportion of the cardiac output available for organs with increased metabolic requirements in emergency situations, including the brain (Yu and Blessing, 1997a; Blessing et al., 1998; Blessing, 2003). Diversion of blood from the cutaneous bed also occurs when the animal is actively exploring the environment, a situation in which the power of hippocampal theta rhythm also increases (Ootsuka et al., 2009).

*The amygdaloid complex and cutaneous blood flow*
Our present study demonstrates that destruction of the amygdaloid complex in the rat substantially impairs SCVARs. After bilateral acute injections of muscimol and one week after bilateral ibotenic acid lesions, the SCVAR response was reduced by approximately 50%. There was no significance difference in SCVAR index between muscimol and ibotenic treatments. Vehicle injections into the amygdaloid complex did not affect the SCVAR index. Our results support our previous demonstration of the importance of the amygdala in mediating the ear pinna blood flow response to salient stimuli in rabbits (Yu and Blessing, 1997b; Yu and Blessing, 1999). Amygdaloid neural circuitry is very important in the regulation of vigilance and in the detection of potentially dangerous (salient) external environmental events (Wan and Swerdlow, 1997; Li et al., 2004; Herdade et al., 2006; Vinkers et al., 2010). Since cutaneous vasoconstriction is a prominent component of the unconditioned autonomic response to a salient event (Yu and Blessing, 1997b; de Menezes et al., 2009), this particular autonomic response is substantially impaired by inactivation of amygdaloid function.

Many previous studies have documented the role of the amygdala in mediating behavioral and autonomic cardiovascular responses to conditioned stimuli (Iwata et al., 1987; LeDoux et al., 1988; LeDoux, 2000; Holahan and White, 2004). In addition, studies show that stressful stimuli, including restraint and air jet stress, increase the expression of Fos protein in the amygdaloid complex (Dayas et al., 1999; Dayas and Day, 2002). There are also many published studies demonstrating variable cardiovascular changes elicited by electrical or chemical stimulation of the amygdaloid complex (Hilton and Zbrozyna, 1963; Cox et al., 1987; Gelsema et al., 1987; Iwata et al., 1987; Brown and Gray, 1988). The amygdaloid complex is also
prominent in functional MRI studies of involving anxiety and fear in humans (Adolphs et al., 1995; Phelps and LeDoux, 2005; Liao et al., 2010).

In contrast, there are relatively few published investigations providing direct evidence that functional inactivation of the amygdaloid complex substantially alters autonomic cardiovascular responses to unconditioned stimuli (loud noises, restraint). Galeno et al. (1984) reported reductions in the amplitude of stress-induced arterial blood pressure increases elicited by 110 db noise after bilateral electrolytic lesions of the amygdala. Kubo et al (2004) reported similar reductions in the amplitude of arterial blood pressure increases elicited by restraint stress after bilateral muscimol injections into the amygdala. On the other hand, Fortaleza and colleagues (2009) found that synaptic blockade in the medial amygdala had little or no effect on blood pressure and heart rate response to restraint stress. Other investigators have demonstrated that the hypothalamic-pituitary-adrenal axis response to stressful stimuli is reasonably normal after amygdaloid lesions (Carter et al., 2004), but the stress induced hyperthermia response is reduced (Vinkers et al., 2010). Startle reactions were preserved after medial amygdaloid lesions (Kemble et al., 1984).

Our present study does not formally report behavioral indices in the ibotenic acid lesioned rats. On simple visual inspection, the rats with chronic bilateral ibotenic acid lesions appeared to exhibit reasonably normal behaviors, but the animals were clearly more active than normal Sprague-Dawley rats. As an example, when transferred to a small plastic bucket on the weighing scale, the lesioned rats kept moving around, pushing up against the lid, so that it was difficult to obtain a steady reading.

The lesions resulting from the ibotenic acid injection were extensive, involving the whole amygdaloid region and, usually, the neighboring piriform cortex, but
sparing the bed nucleus of the stria terminalis and the ventral hippocampus. When the muscimol injection sites were visualized using the HRP reaction product, the injected area included all the amygdaloid nuclei, but there was no spread to the piriform cortex. Neither the acute bilateral muscimol nor the chronic bilateral ibotenic acid procedure completely abolished the cutaneous blood flow response. Similarly, the cardiovascular response to conditioned fear stimuli is substantially reduced but not completely abolished by inactivation of amygdaloid function (LeDoux et al., 1986).

The descending neuroanatomical pathway for the amygdaloid-mediated response to conditioned fear includes the dorsomedial hypothalamus and the midbrain periaqueductal grey (LeDoux et al., 1988). The corresponding descending pathway for the natural unconditioned autonomic response to salient stimuli is not yet established, but the dorsomedial hypothalamic nucleus and the medullary raphe nuclei are likely to be involved (Nalivaiko and Blessing, 2001, 2002). The preservation of a degree of the vasoconstrictor response to salient stimuli after extensive inactivation of amygdaloid function emphasizes the importance of other forebrain pathways, including the medial prefrontal cortex, the ventral hippocampus and the bed nucleus of the stria terminalis (Radley and Sawchenko, 2011) in the mediation of autonomic responses to salient stimuli. Projections from the amygdala to the hypothalamus and lower brainstem are predominantly ipsilateral (Price and Amaral, 1981; Gray et al., 1989; Blessing et al., 1991), a factor that could contribute to the greater effect of bilateral compared to unilateral amygdaloid lesions.

Inactivation of amygdaloid function reduced the SCVAR index for all of the 6 salient stimuli, in both the acute and the chronic experiments, so that the tail vasoconstrictor response to perception of a salient stimulus is not restricted to a particular sensory modality. In our previous study with bilateral muscimol
inactivation of the amygdala in rabbits (Yu and Blessing, 2001), ear pinna vasoconstriction could still be elicited by application of a normally painful stimulus (forceful pinching of the ear pinna). Even in anesthetized rabbits, nociceptive stimulation vigorously reduces cutaneous blood flow, by a brain pathway that descends to the spinal cord via a synapse in the medullary raphe nuclei (Blessing et al., 1998; Blessing and Nalivaiko, 2000). The hypothalamic and/or brainstem neural circuitry mediating the nociceptive cutaneous vasoconstrictor response might form part of the descending pathways for the cutaneous vasoconstrictor response to salient stimuli that is mediated by amygdaloid neural circuitry.

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**Figure Legends**

Figure 1.
Tail artery blood flow Doppler signals recorded approximately 30 min after the rat was transferred into the experimental cage. Either vehicle (A) or muscimol (B) was injected bilaterally into the amygdaloid complex, with the procedure commencing at the points marked by the small vertical arrows.

Figure 2.
Tail artery blood flow Doppler signals recorded approximately 1 week after bilateral injection of vehicle (A) or ibotenic acid (B) into the amygdaloid complex. Each record begins approximately 30 min after the rat was transferred to the experimental cage.

Figure 3.
Pulsatile tail artery blood flow signal recorded during a single tap (small vertical arrow) on the outside of the closed wooden box, at least one week after injection of (A) vehicle, or (B) unilateral ibotenic acid, or (C) bilateral ibotenic acid into the amygdaloid complex of 3 different rats.

Figure 4.
Group data (mean±sem) showing the SCVAR index produced by each of the series of 6 standardized salient stimuli. A, after acute bilateral injection of vehicle (unfilled bars, n=7 rats) or muscimol (filled bars, n=6 rats). B, one week after bilateral injections of vehicle (unfilled bars, n=6 rats) or ibotenic acid (filled bars, n=6 rats). ¶¶ p<0.01 versus vehicle.
Figure 5.
Photographs taken from coronal sections of a rat brain after intra-amygdala injections of HRP with the muscimol solution (A) or fluorescent beads (B). Arrows in (A) indicate the tract made by the guide cannulae and arrow in (B) indicate the fluorescent beads at the injections site. Figure 5C, shows the effect of chronic vehicle injections and fig 5D, represents the effect of ibotenic acid in the amygdaloid complex. OT optic tract; CE central nucleus of the amygdala; MeA medial amygdala; LA lateral amygdala; BA basal amygdala; Pir piriform cortex.

Table 1.
Effects of bilateral injections of vehicle or muscimol into the amygdaloid complex in conscious freely moving rats. Grouped data (mean±sem) for mean blood flow and coefficient of variation before and after injection, and the overall SCVAR index for combined stimuli after injection. * not significantly different from vehicle pre-injection value; ns, not significantly different from the corresponding pre-injection value, P>0.05; ¶¶, significantly different from the pre-injection value P<0.01; §§ significantly different from the value after injection of vehicle P<0.01; n=7 rats for vehicle and n=6 for muscimol.

Table 2.
Effect of unilateral or bilateral injections of vehicle, unilateral ibotenic acid or bilateral ibotenic acid into the amygdaloid complex. Group results (mean±sem) show the mean blood flow, the coefficient of variation and the overall SCVAR index for combined stimuli, recorded at least one week after the injections. ns, not significantly different from vehicle, P>0.05; ¶ significantly different from vehicle, P<0.05; ¶¶
significantly different from vehicle, P<0.01; ** significantly less than SCVAR index after unilateral ibotenic acid, P<0.01; § not significantly different from the SCVAR index after acute bilateral injection of muscimol (see muscimol result in Table 1). n=12 rats for vehicle and n=6 rats for unilateral ibotenic acid and n=6 rats for bilateral ibotenic acid.
Table 1:

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Table 2:

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FIGURE 1

[Graph showing changes in tail artery Doppler blood flow signal (cm/s) with Vehicle and Muscimol administrations.]

A

B

Vehicle

Muscinol

5 min
FIGURE 2
FIGURE 3

A. One week after vehicle

B. One week after unilateral ibotenic acid

C. One week after bilateral ibotenic acid
FIGURE 4

A. Acute muscimol or vehicle injections

B. Chronic ibotenic acid or vehicle injections

SCV/AR index (%)

Tap  Sound  Drop  Move  Open  Pinprick