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Correlational Analysis of Central Noradrenergic Neuronal Activity and Sympathetic Tone in Behaving Cats

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The activity of the noradrenergic (NA) neurons of the feline locus coeruleus complex (LCx) were correlated with changes in peripheral sympathetic tone in behaving cats. LCx NA neurons exhibit stereotypical changes in discharge rate across behavioral states, falling virtually silent during paradoxical sleep (PS). Simultaneous recordings from LCx NA neurons and the cervical sympathetic trunk demonstrated a tonic reduction in nerve activity during PS as well. LCx NA neurons were also found to fall silent during induction of the scruff immobility reflex and significant pupillary miosis was seen during this behavior as well. These data support the hypothesis that NA neurons in the brain are a central analogue of the peripheral sympathetic system and demonstrate that the two systems operate in an integrated fashion in the behaving cat.

INTRODUCTION

Noradrenergic (NA) neurons rank among the most intensively studied group of neurons in the central nervous system. In the cat, NA neurons are heterogeneously distributed throughout the locus coeruleus complex (LCx, operationally defined as nuclei locus coeruleus, subcoeruleus, parabrachialis medialis and lateralis, and Kolliker–Fuse)[9,22,23,26,27,30,41]. Within the LCx, there exists a subpopulation of neurons whose members exhibit stereotypical variation in their mean and absolute discharge rates across behavioral states[18,19,35–37]. In unanesthetized animals, these neurons typically fire at their highest rates during active waking (AW), at lower rates during quiet waking (QW), still lower during slow wave sleep (SWS), and fall virtually silent during paradoxical sleep (PS).

Several lines of evidence support the hypothesis that these 'PS-off' cells are NA. In the rat, PS-off cells are restricted to the homogeneously NA locus coeruleus[2]. Clonidine, an adrenergic agonist which inhibits NA neurons in the anesthetized rat[8,38], also inhibits PS-off cells in the behaving cat[33]. Finally, with the exception of the Kolliker–Fuse (KF) nucleus, PS-off cells have been recorded in all the nuclei of the LCx in which NA neurons are found[18,19,35–37]. In order to further document the correlation between the distribution of NA neurons and PS-off cells in the cat, we have also included in the present report our observations upon the spontaneous activity of KF neurons across behavioral state.

The major question which this study addresses is the extent to which central NA neuronal activity covaries with peripheral sympathetic tone in the behaving cat. While central NA neurons have often been suggested to behave as a 'central analogue of peripheral sympathetic neurons'[1,6,12] this is the first report of direct recordings of central NA neurons and peripheral sympathetic activity in behaving animals.

Some of these data have been previously presented in preliminary form[29,31,32].

MATERIALS AND METHODS

Experiments were performed on 12 adult, female cats, ranging in weight from 2 to 4.5 kg, kept on a 12:12 light/dark cycle. All animals received routine

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physical and neurological examinations; the animals maintained excellent health throughout the duration of the experiments, which lasted 1–3 months for any given animal. Under halothane anesthesia and aseptic conditions, cats were implanted with standard electrodes for recording the electroencephalogram (EEG), electro-oculogram (EOG) and nuchal electromyogram (EMG). In 8 animals, a bipolar macroelectrode was stereotaxically lowered into the right dorsal lateral geniculate nucleus (A = 6.0, L = 11.0, V = 3.3) to record pontine-geniculate-occipital (PGO) spikes.

An adaptation of the microwire technique described by Harper and McGinty[16] was used to record from single neurons in the LCx of behaving cats. A miniature microdrive, with two cannulae separated in the sagittal plane by 1.5 mm, was stereotaxically implanted at a 30° angle from the vertical, thereby aligning the cannulae parallel to the bony tentorium. The microdrive was stereotaxically lowered until the tip of the rostral cannula was 6 mm above the intended recording site and cemented in place. Two bundles of formvar-coated nichrome microwires (California Fine Wire, Stablohm 675, stress-relieved) were then lowered through the two cannulae to a point 5 mm past their tips and cemented with cyanoacrylate to the microdrive; in this manner, the distal ends of the microwire bundles were placed 1 mm above the intended recording site. Each bundle consisted of 6 wires each, either in equal combinations of 32 and 62.5 μm or all 32 μm diameter. The microdrive was equipped with an 0–80 screw by which it could be advanced in steps as small as 37.5 μm. In 9 animals, microwire electrodes were aimed at the locus coeruleus proper (P = 3.0, L = 3.0, V = –3.0); two were aimed somewhat more laterally at the parabrachial nuclei (PBL and PBM), (P = 3.0, L = 4.0, V = –3.0); one animal had an implant aimed at the Kolliker–Fuse (KF) nucleus (P = 3.0, L = –5.0, V = –4.0).

The activity of the cervical sympathetic trunk was recorded by means of a chronic nerve cuff, implanted in a second surgical procedure. The cat was placed in dorsal recumbency and the ventral surface of the neck prepared for aseptic implantation of the nerve cuff. A longitudinal skin incision was made on the ventral midline and the sternohyoideus muscle was sharply dissected at the midline to expose the underlying trachea. Blunt dissection was used to locate the right carotid sheath, which was carefully dissected to expose the vagosympathetic trunk. The trunk was gently retracted and the sympathetic trunk detached from the vagus nerve by delicate blunt and sharp dissection of the connecting epineural fascia, taking care not to disturb its blood supply. Once the cervical sympathetic trunk was freed of the vagus, the nerve cuff was carefully opened, the nerve gently placed inside and the cuff tied in place. The nerve was irrigated with dexamethasone, and the leads of the nerve cuff were soldered to preplaced wires lying in the neck which connected with two pins in the head plug.

Construction of the nerve cuff was modelled after the descriptions of Hoffer[20] and Davis et al.[11], to which the reader is referred for further details of cuff manufacture. Briefly, three 32 μm diameter nichrome wires were sewn circumferentially into the wall of a 1.5 cm length of silastic tubing (i.d. 1.1 mm) at 0.4 mm intervals. Construction was such that wires covered the entire inner circumference of the tubing, except for a longitudinal line which was then slit and later opened to permit placement of the nerve inside the cuff. The two wires at the ends of the cuff were grounded together for optimal rejection of (common) EMG activity when recorded differentially against the central lead of the cuff.

Data collection

Data were collected by means of low noise cable (Microdot) attached to a 24-pole commutator (BRS/LVE) which was seated within a custom-designed counterbalance mechanism which permitted relatively unimpeded movement throughout the cage. Macroelectrode activity was led directly to a polygraph (Grass, model 78D); up to two channels of macropotential activity were fed through an FM converter (Vetter) and recorded on magnetic tape (Sony, model TC-338-4). The microwire output was connected to a differential AC preamplifier (Grass, P15) which amplified the signal ×1000 (low filter: 300 Hz, high filter: 10,000 Hz). A 'silent' microwire from the opposite bundle was used as the indifferent electrode. Selected portions of data were recorded directly on tape along with concurrent macropotential activity. The signal was continually monitored on an oscilloscope (Tektronix, RM 565) and was also led

to a window discriminator (WPI, 120) which provided TTL pulses to (a) deflect a polygraph pen, (b) trigger an individual sweep of a digital oscilloscope (Tektronix, 5223), and (c) provide digital input to a microprocessor for high-speed data analysis. The digital oscilloscope was equipped with a delay circuit which allowed viewing the entire waveform of each action potential; constant monitoring of the action potential waveform insured that there was no profound variation in either spike height or configuration over the course of the recording sessions.

During a typical recording session, the animal was placed in a well-lit, shielded and sound-attenuated cage, equipped with a one-way mirror, with food and water available ad libitum. The animal was connected to the counterbalanced cable assembly and data was recorded across several behavioral states, including at least two complete sleep–wake cycles. All data was recorded on polygraph paper; for all neurons, at least 60 s of data for each of 4 behavioral states (below) was recorded on magnetic tape for later analysis.

In a pilot study we found that LCx PS-off cells also fell silent during the scruff immobility reflex (SIR). Therefore, we examined the activity of 18 LCx neurons during this behavior. SIR was induced by grasping the cat firmly by the nape of the neck and lifting it off the floor of the cage. Simultaneously, a microswitch was depressed, causing a pen to deflect on the polygraph, thereby marking both onset and duration of SIR.

The activity of the cervical sympathetic nerve was led from the plug on top of the cat's head to the commutator and then to a high impedance preamplifier (Grass, P-15), where the signal was amplified (100×) and filtered (low filter: 100 Hz; high filter: 10,000 Hz). From there, the signal was led directly to one channel of the polygraph, as well as to an oscilloscope.

In order to (indirectly) assess sympathetic tone during induction of the SIR, pupil diameter was examined in 17 normal cats. Three naive observers were used to rate the changes in pupil diameter during SIR. Each observer was asked to report only unambiguous changes, be they constriction or dilatation. Each report of constriction was scored at +1, and each report of dilatation was scored as −1. The scores were summated; if all 3 observers reported constriction, the total score for that animal was +3, etc. While these naive observers recorded their observations of pupil diameter, the experimenter rated the degree to which the animal exhibited the SIR; for in preliminary experiments, we found that SIR can be evoked only in some adults (while it appears to be robust in all neonates). Consequently, animals were divided into two categories, those who exhibited profound SIR, and those who struggled upon handling.

For all neurons which had stable, well-isolated action potentials, we recorded single unit activity during 4 well-characterized behavioral states[40]. Episodes of AW were induced by introducing a mouse into the litter pan at the bottom of the cage; the mouse was inaccessible to the cat but the presence of the mouse reliably aroused the cat. QW, SWS and PS occurred spontaneously.

Data analysis

Behavioral states were scored by standard criteria on the basis of EEG, EMG and EOG data, as well as behavioral observations noted at the time of data collection. Mean firing rate across behavioral state of all neurons was determined by off-line analysis on a Z-80 based microprocessor. Sixty second epochs of neuronal activity in each behavioral state (AW, QW, SWS and PS) were used to compute mean firing rate.

Neurons were divided according to their state-related neurophysiological profiles (see Results) into PS-off cells and non-PS-off cells. The mean firing rates during 4 behavioral states for the entire population of PS-off cells were analyzed as a group, to determine if there were statistically significant differences in discharge rate between states. Because the mean firing rates of individual PS-off cells were not normally distributed (see Fig. 3), we transformed the data using the formula $x_t = \log(x_s + 1)$, where x_s represents the mean firing rate of each neuron during a given behavioral state and x_t represents the transformed rate. These data were then subjected to a single classification analysis of variance and back-transformed to obtain the reported means and levels of significance.

Anatomical localization of electrodes

At the conclusion of the experiments, the animals were deeply anesthetized with pentobarbital and 0.7 mA of cathodal current was passed through several

(2–4) microwires for 1.5 s; in this manner, a small amount of iron was deposited at the end of the electrode tracts. The animal was then perfused transcardially with one pass of heparinized saline (37 °C), followed by 5% potassium ferrocyanide dissolved in 10% formalin. The brains were removed, blocked and cut into 40-μm thickness sagittal sections on a freezing microtome. Sections were counterstained with neutral red to enhance visualization of the Prussian blue reaction product. The location of the tips of the microwires at the time of recording individual units was then reconstructed on standard sagittal sections of the brainstem.

RESULTS

State-related activity of LCx neurons

The data are extracellular recordings of 131 neurons localized within the dorsolateral pontine tegmentum of 12 cats. Based upon their state-related neurophysiological profiles, these neurons were divided into two categories. (a) PS-off cells (n = 53) which exhibited stereotypical variation in mean discharge rate across behavioral states, firing at high rates during AW, less during QW, still less during SWS and falling virtually silent during PS (Figs. 1–4); and (b) non-PS-off cells (n = 78), a category including all neurons which did not exhibit the stereotypical pattern of variation in mean discharge rate across behavioral states characteristic of PS-off cells. This latter group comprised many different types of neurons, including those which tonically fired slowly across all states, those firing fast across all states, those with respiratory related discharge and even some which were silent in all states except for PS.

PS-off cells fired most in AW (2.08 ± 0.31; mean ±

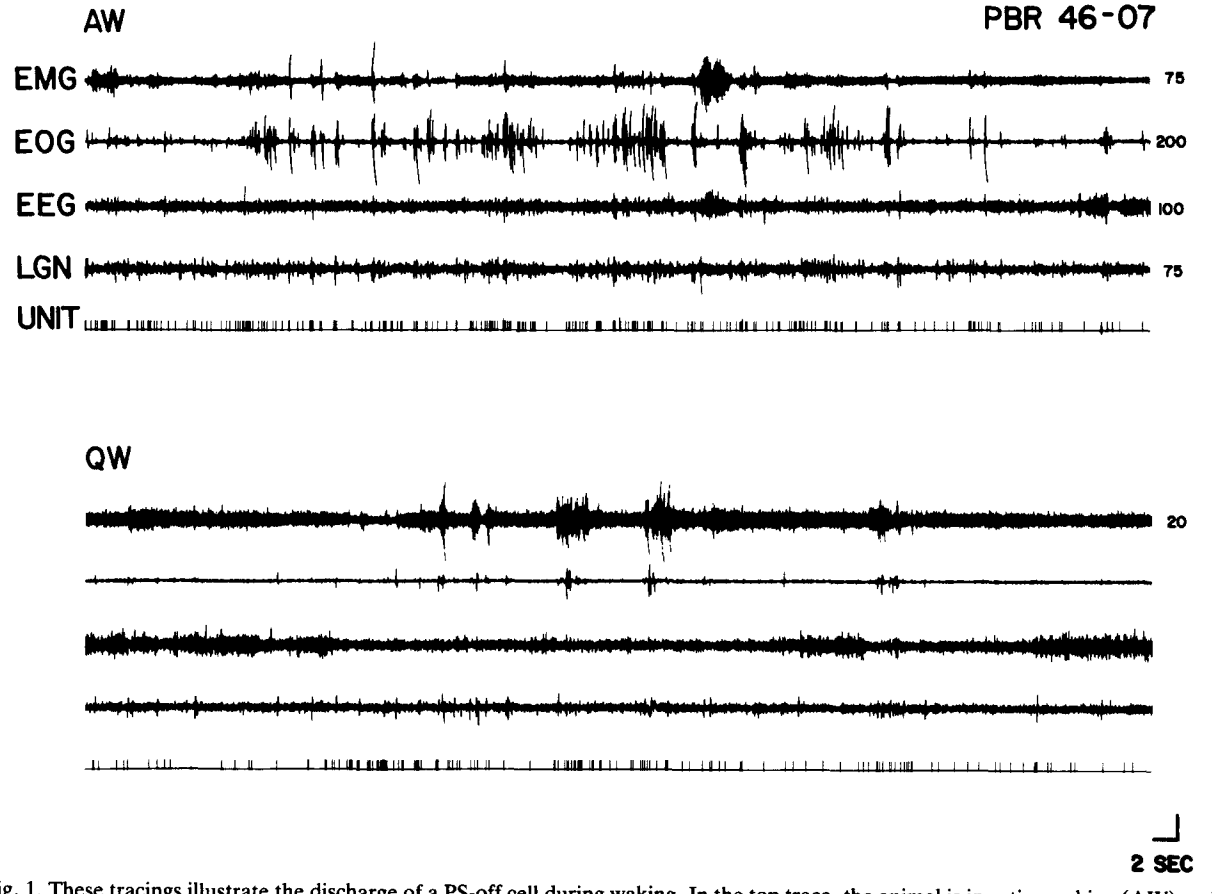

Fig. 1. These tracings illustrate the discharge of a PS-off cell during waking. In the top trace, the animal is in active waking (AW) and the neuron fires at relatively high rates, with moderate lability of discharge across time. The lower trace shows the same parameters during quiet waking (QW), with the neuron firing at a slightly lower rate. In this and all subsequent figures, the vertical calibration is in μV. Abbreviations: EMG, electromyogram; EOG, electrooculogram; EEG, electroencephalogram; LGN, lateral geniculate nucleus; UNIT, neuronal discharge.

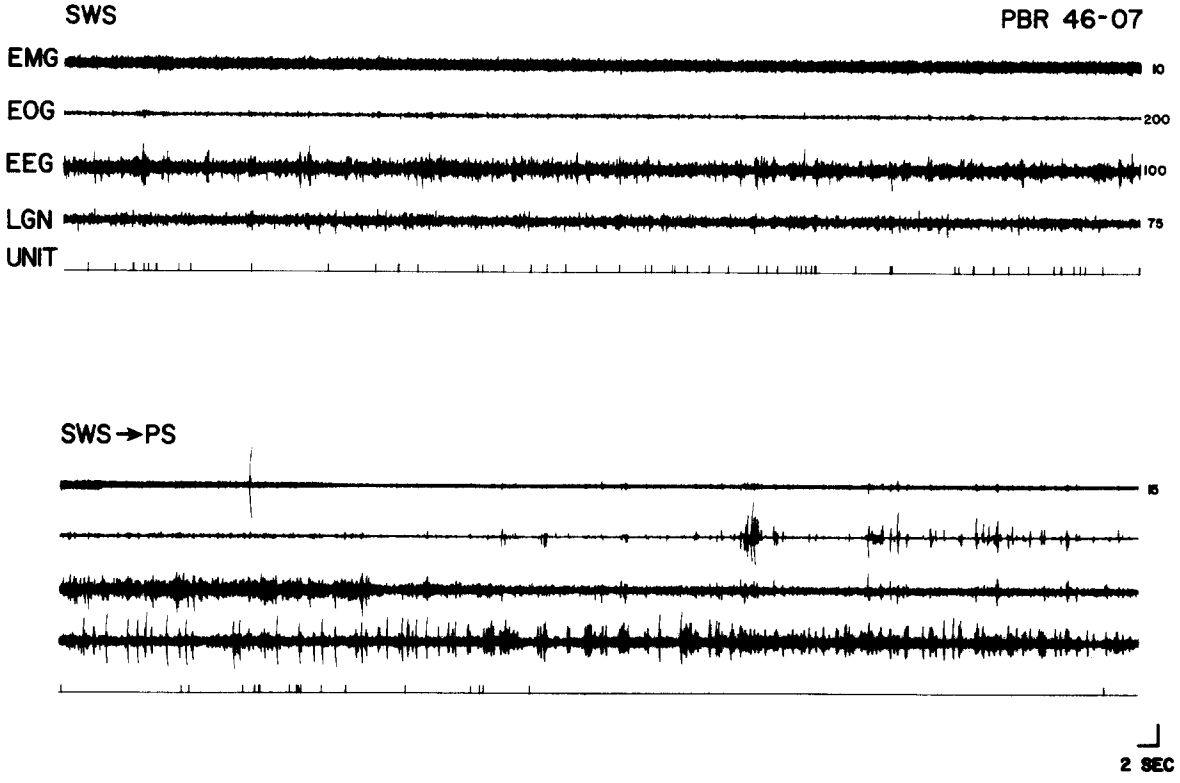

Fig. 2. These tracings are from the same neuron as in Fig. 1. Note in particular the further reduction in neuronal activity during SWS and the gradual onset of neuronal silence as PS evolves, with only rare firings during the subsequent episode. Abbreviations as in Fig. 1.

S.E.), less so in QW (1.47 ± 0.16), still less in SWS (0.67 ± 0.11) and fell virtually silent during PS (0.04 ± 0.02). An individual LCx PS-off cell is shown during each of these 4 states in Figs. 1 and 2, and the firing rates of each of these 53 PS-off cells across behavioral states is shown graphically in Fig. 3. These firing rates were not normally distributed around the mean, but rather were skewed towards the lower end of the scale, as can be seen most clearly for the rates during AW. For this reason, statistical comparisons between the group means were performed upon the transformed data, as detailed in Materials and Methods, and back-transformed to obtain the reported means and standard errors. The differences between the group means are all statistically significant and are shown graphically in Fig. 4.

PS-off cells invariably discharged at their highest rates during AW. Neuronal activity did not appear to be consistently associated with any individual motor act during waking, although the animals were capable of, and indeed exhibited, a variety of motor be-

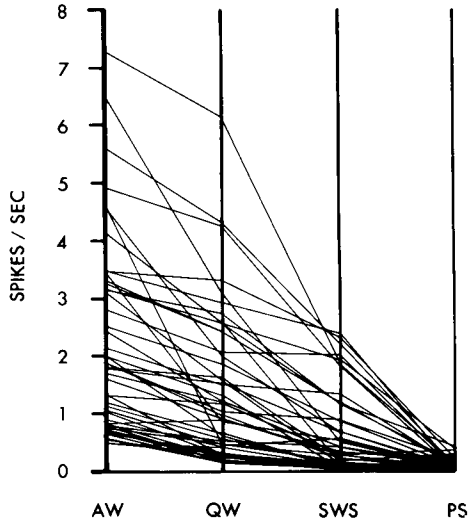

Fig. 3. This graph shows the individual firing rates of 53 LCx PS-off cells across 4 behavioral states. The firing rates were computed from a 60-s sample of unit activity recorded during each state. Note that the individual firing rates of LCx neurons are not normally distributed around the mean (clearest in AW).

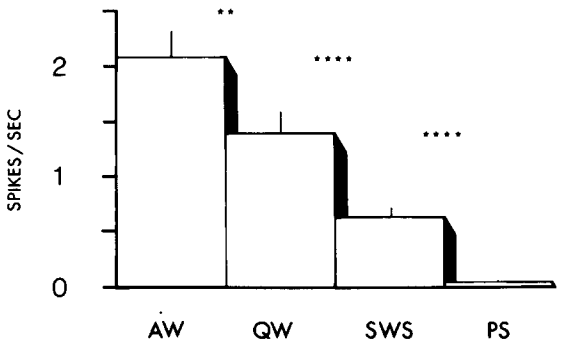

Fig. 4. This graph shows the group means and S.E.M. for all 53 LCx PS-off cells recorded across 4 behavioral states. Because the individual firing rates of LCx neurons are not normally distributed around the mean (see Fig. 3), the data were transformed into logarithmic form, means computed on the transformed data and then back-transformed to obtain the data presented in this graph. This graph shows that the differences in mean firing rate of all LCx PS-off cells across states are statistically significant (single classification analysis of variance). Key: ** $P < 0.01$; **** $P < 0.001$.

haviors during the testing protocol. During QW, these neurons fired with greater regularity than during AW, in addition to the reduction in mean firing rate. As the animal entered SWS, there was a gradual reduction in the mean firing rate of these neurons. At the transition to PS, neuronal activity became further reduced; most PS-off cells exhibited no spontaneous activity during PS at all. This reduction in neuronal activity was maintained throughout each episode of PS; unit activity always returned at the end of each bout of PS. On the basis of these behavioral neurophysiological data in conjunction with our previous pharmacological observations[33], we established minimal criteria for inclusion in this category: mean discharge rate of <0.50 Hz during PS and at least 30 s of total silence during PS lasting 2 min or more.

It should be noted that PS-off cells were almost always found interdigitated with non-PS-off cells throughout the LCx; indeed, PS-off cells and non-PS-off cells were frequently encountered side by side, and it was occasionally possible to record from a PS-off cell and a non-PS-off simultaneously from the same electrode.

The scruff immobility reflex (SIR) was induced by grasping a cat by the nape of the neck and lifting it off the floor of the cage. During SIR, cats exhibit contraction of the limbs, with the tail curving under the body. Furthermore, cats were less responsive to environmental stimuli during this behavior, whether they are innocuous or noxious. We found 7/10 PS-off cells to be silent during SIR (Fig. 7), while 0/8 non-PS-off cells fell silent following the same protocol. These data show that SIR represents a second behavioral state in which LCx PS-off cells fall silent.

Anatomical localization of PS-off cells

The reconstruction of recording sites was accomplished by examination of the electrode track and the iron deposition at the tip of the track, visualized as Prussian blue reaction product. PS-off cells were re-

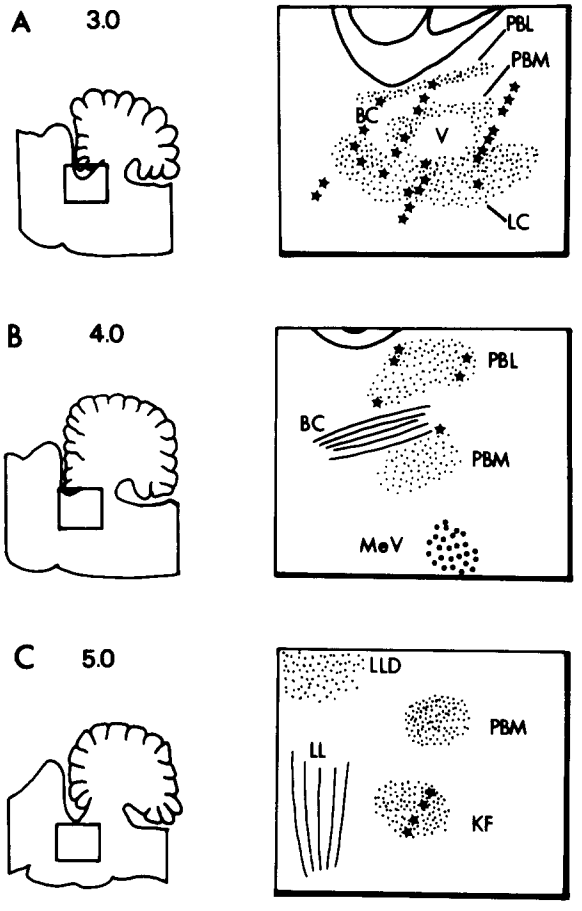

Fig. 5. Composite drawing of the locations (stars) of 37 LCx PS-off cells reconstructed from their electrode tracts, drawn on standard parasagittal sections of the brainstem taken at 3 levels (3.0, 4.0 and 5.0 mm off the midline). In each figure, the area of detail (right) is shown in relation to the brainstem as a whole on the left. Abbreviations: BC, brachium conjunctivum; LC, nucleus locus coeruleus; LL, lateral lemniscus; LLD, dorsal nucleus of lateral lemniscus; KF, Kolliker–Fuse nucleus; MeV, mesencephalic nucleus of the trigeminal complex; PBL, nucleus parabrachialis lateralis; PBM, nucleus parabrachialis medialis; V, tract of the trigeminal complex.

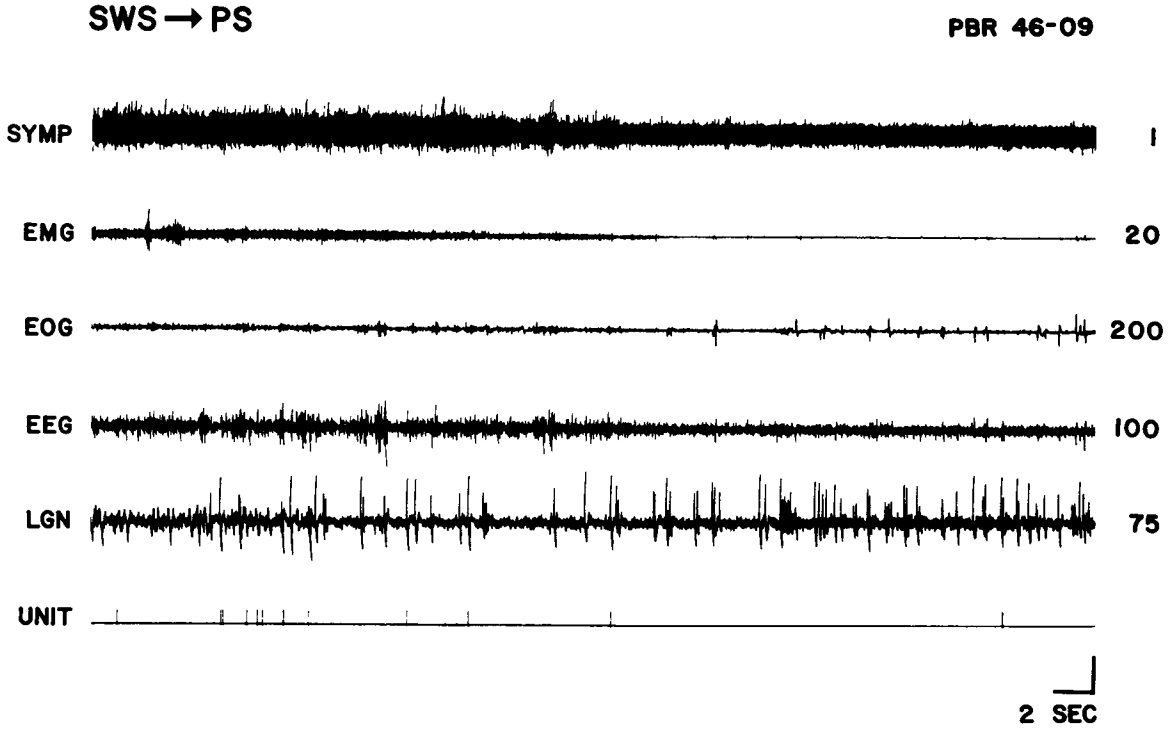

Fig. 6. Cervical sympathetic tone (SYMP), as measured by whole nerve recording with a chronic nerve cuff during the transition to PS. Note the gradual reduction in tone as the transition to PS progresses and the tonic reduction of sympathetic tone during PS. Abbreviations as in Fig. 1.

corded throughout the LCx, including the nuclei locus coeruleus, parabrachialis medialis and lateralis, and Kolliker–Fuse (Fig. 5). Because this is the first report of PS-off cells in the Kolliker–Fuse nucleus, we compared the state-related group mean firing rates of neurons in our larger sample of more medially lying PS-off cells with those in this laterally lying cell group. There were no significant differences between the two.

PS-off cell activity and sympathetic tone

Successful recordings of the activity of the right cervical sympathetic (cSYM) trunk across behavioral state were obtained in 3 animals. In all 3, we found cSYM activity to be tonically reduced during PS (Fig. 6). This reduction lasted throughout each epoch of PS; following arousal from PS, the cSYM activity returned to that observed during SWS.

Several means were used to confirm that the recorded activity was that of sympathetic nerves rather than changes in tone of surrounding muscles. First, in several animals, we observed ipsilateral prolapse of the nictitating membrane and marked miosis and ptosis (Horner's syndrome) immediately upon recovery from surgery. This syndrome is a classic sign of damage to the sympathetic innervation of the eye, sug-

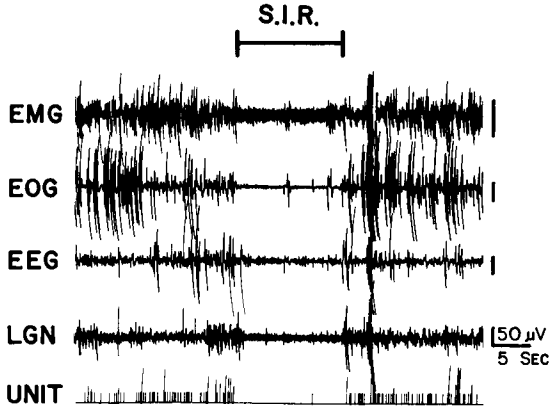

Fig. 7. Polygraph record of physiological parameters recorded during the scruff immobility reflex (SIR). Note the profound silence of the neuron during SIR. Also, muscle tone (EMG), although reduced, is maintained throughout the epoch of SIR. Abbreviations as in Fig. 1.

gesting that we had damaged the cSYM trunk during surgical implantation of the nerve cuff. In these animals, we never saw the tonic reduction of recorded activity which we observed in those animals without Horner's syndrome. Indeed, recordings from these animals showed no change in activity regardless of behavioral state, with the exception of movement artifacts during AW. Secondly, following a period of 1–2 weeks, all 3 of our successful nerve cuff implants failed, probably due to mechanical damage by the nerve cuff of the cSYM trunk. These animals exhibited acute onset of ipsilateral Horner's syndrome. Simultaneously, the recordings no longer demonstrated tonic reductions of activity during PS, as they had prior to the onset of Horner's syndrome. Finally, at the conclusion of several episodes of PS, we noted that the return of nuchal EMG tone preceded the return of activity in our nerve cuff recordings by several seconds; although it possible that tone returns at different times in different muscles at the end of PS, such dramatic differences as we noted are unlikely. Consequently, we feel that our recordings were in fact obtained from the cSYM trunk and that we have therefore demonstrated that cSYM activity is tonically reduced during PS.

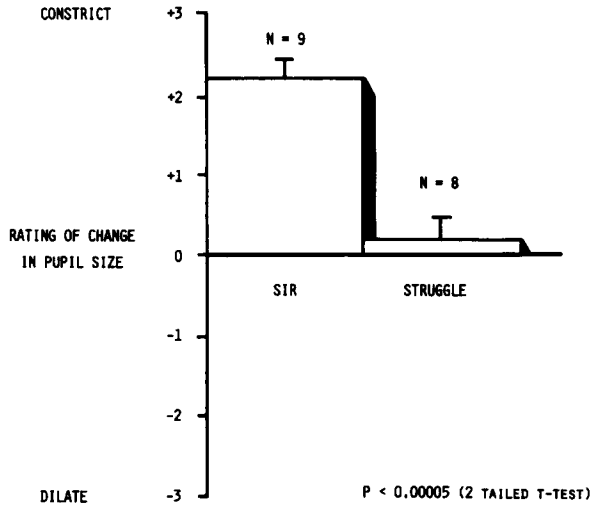

Fig. 8. The effects of the scruff immobility reflex (SIR) on pupil diameter are shown graphically in this figure. Each of 3 naive observers was asked to determine if SIR had an unambiguous effect upon pupil diameter. Each observer's answer was converted into a rating scale (−3 to +3), which was used to determine the statistical significance of the effect upon 9 cats who exhibited profound SIR and 8 cats who struggled upon handling (2 tailed *t*-test).

We utilized an indirect measure of sympathetic tone, pupil diameter, to quantify autonomic responses during SIR. Significant miosis was consistently noted in animals exhibiting strong behavioral SIR as compared to animals who struggled upon handling (Fig. 8). That this effect is robust is indicated by the fact that the observers were instructed to rate changes in pupil diameter only if they were unambiguous; subtle changes in diameter were to be rated as no change.

DISCUSSION

In the present report, simultaneous recordings from central NA neurons and peripheral sympathetic nerves were used to study their covariation across behavioral states. Furthermore, the anatomical distribution of PS-off cells within the LCx was examined, as was the activity of these neurons during SIR.

The evidence that these feline LCx PS-off cells are NA derives from several sources. PS-off cells are heterogeneously distributed throughout the feline LCx, as are NA neurons as demonstrated by numerous anatomical studies[9,22,23,26,27,30,41]. Furthermore, these cells can be found in all of the nuclei of the feline LCx in which NA neurons are located, including the KF (present report). In the rat, PS-off cells are restricted to the locus coeruleus[2] and in this species the nucleus is homogeneously NA[10,15,17,28,39]. The α_2 adrenoceptor agonist clonidine inhibits NA neurons in the anesthetized rat when applied either microiontophoretically, intraventricularly or intravenously[8,38]. We have recently shown that LCx PS-off cells are potently inhibited by intravenous clonidine in the unanesthetized cat[33]. These anatomical, pharmacological and behavioral neurophysiological data provide convergent evidence that LCx PS-off cells are NA.

Our approach to studying the covariation of NA LCx PS-off cells and peripheral sympathetic tone has made use of the spontaneous variation in these physiological parameters which accompanies changes in behavioral state. The first direct evidence that sympathetic tone was reduced during natural PS was obtained by Baust and his colleagues, who recorded the activity of the renal nerve across behavioral states[3–5]. We have extended these findings by showing that the activity of the cervical sympathetic trunk, like that of the renal nerve, is tonically reduced during PS.

These observations in intact animals are supported by results obtained in decerebrate preparations, which exhibit periodic episodes of muscular atonia which mimic those seen during normal PS[24]. Renal nerve activity is tonically reduced during 'decerebrate PS'[14,21], as is the activity of the cardiac and splanchnic nerves and the lumbar sympathetic chain[14]. Surprisingly, sympathetic vasoconstrictor fibers innervating the gastrocnemius muscle (and possibly other muscles as well) increase their activity during 'decerebrate PS'[14], as had been suggested by Reis et al.[34] on the basis of blood flow redistribution during natural PS. This anomaly aside, it seems clear that there exists a tonic reduction in the activity of sympathetic nerves innervating viscera during PS.

As we have documented, both cervical sympathetic tone and central NA neuronal activity are tonically reduced during PS. We were unable to obtain adequate recordings of cervical sympathetic activity during more active waking periods and therefore cannot comment directly upon the activity of the peripheral sympathetic system during waking. However, studies dating back to Cannon[7] have consistently associated increased sympathetic outflow with arousal, especially in situations in which an animal is presented with prey, such as our AW paradigm. Indeed, the sympathetic system is classically described as being involved in 'fight or flight' responses, and it can reasonably be inferred that peripheral sympathetic nerves are highly active during AW.

We have shown that LCx PS-off cells fall silent during SIR. We found significant pupillary miosis during this behavior as well. These observations are consistent with the notion that cervical sympathetic tone is reduced during SIR. However, it should be noted that the pupil is not solely under sympathetic control; indeed the observed miosis could be a result of increased parasympathetic tone, rather than decreased sympathetic tone. Pending more direct evidence, we must be cautious in concluding that sympathetic tone is reduced during SIR; however, the results of this experiment complement our observations on the covariation of LCx neuronal activity and peripheral sympathetic tone across behavioral states.

In summary, we have directly shown that central NA neuronal activity and peripheral sympathetic tone undergo strikingly parallel changes across several behavioral states. These data from simultaneous recordings of individual NA neurons and sympathetic nerves in unanesthetized cats are fully supported by observations in anesthetized rats[12]. These investigators found parallel changes in central NA neuronal activity and splanchnic nerve activity in response to physiological (blood volume) and pharmacological manipulations. Thus, not only do these two systems covary under the well-controlled but relatively unnatural conditions of acute neurophysiology, but also spontaneously in behaving animals.

The data presented herein provide direct support for a concept dating back to Brodie and Shore[6], who in 1957 conceived of central NA neurons as a 'central sympathetic center'. Their hypothesis was later formalized by Amaral and Sinnamon[1], who concluded their insightful review with the speculation that the LCx is a 'central analogue of the sympathetic ganglia with brain as its end-organ'. Whether the observed covariation of central and peripheral 'sympathetic' activity is causal or not remains a question for future study. However, it is apparent that these two important physiological systems, with projections ramifying widely throughout the body and the central nervous system, function as an integrated unit in the maintenance of homeostasis.

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